ASPECTS OF GROWTH HORMONE IN THE PHYSIOLOGY OF SMOLTIFICATION AND SEAWATER ADAPTATION OF COHO SALMON, *ONCORHYNCHUS KISUTCH*

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Aspects of growth hormone in the Physiology of smoltification and seawater adaptation of coho salmon, Oncorhynchus kisutch

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ABSTRACT

Juvenile coho salmon undergo a complex transition of morphological, behavioural and physiological parameters which tranform the freshwater parr to a seawater-adaptable smolt. To investigate the suspected role(s) of growth hormone (GH)in smoltification, a series of experiments were performed. The first set of experiments examined effects of exogenous GH on metabolism during smoltification. In parr and pre-smolts, but not in smolts, GH treatment induced a 2-fold hyperglycemia. No effects were observed on plasma amino acid nitrogen or fatty acid levels. Additionally, there were no consistent effects noted in muscle protein or amino acid nitrogen levels. The results are discussed in terms of tissue sensitivities and teleost metabolic rates.

The major study on smoltification and GH consisted of two seasons of weekly sampling experiments, encompassing smoltification and subsequent desmoltification. Increases in plasma GH levels during smoltification were noted in both studies. These increases were correlated with changes in plasma and tissue metabolite levels, as well as with changes in growth patterns. The significance of these changes and interrelationships are discussed with reference to the dynamic aspects of smoltification, including external influences. Fish undergoing desmoltification also displayed elevated GH levels, although metabolite levels appeared to be reverting to the freshwater state. Functions of GH in these animals are also discussed.

The second phase of the thesis focused on actual seawater adaptation, examining the proposed hypoosmoregulatory actions of GH. Seawater transfer led to rapid and dramatic increases in plasma GH, followed by changes in carbohydrate and lipid metabolism. These changes occur as plasma ion regulation begins. Pretreatment with somatostatin, a biological inhibitor of GH

release, decreased plasma GH levels in the initial 12 hours of seawater exposure, but had no effect on ion regulation. These results show GH acts in the regulatory, as opposed to the adjustive, phase of seawater adaptation. Possible implications of the hyperlipidemia and depletion of body glycogen stores and the roles of GH in mediating these changes in metabolism are discussed, as is the involvement of other hormones, in terms of successful seawater adaptation and growth.

ACKNOWLEDGEMENTS

I wish to dedicate this thesis to my wife, Maureen, without whose constant love and support I would not have been able to continue.

I would like to extend my sincere gratitude to my Supervisor, Dr. Brian A. McKeown, for his continual support, interest and guidance throughout my research. His contributions, both as a professional and as a friend, were very important features of this work.

I would also like to thank my mother and father, who never doubted that this day would happen.

Finally, I wish to extend my heartfelt thanks to the many fellow graduate students, staff and faculty in the Department who made my graduate career such an enjoyable experience. To those special people who have become lifelong friends, I share this thesis with you.

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GENERAL INTRODUCTION

The coho salmon (*Oncorhynchus kisutch*) is a member of the sub-family *Salmoninae*. This sub-family, and particulary two genera, the *Salmo* (trout and Atlantic salmon) and the *Oncorhynchus* (Pacific salmon), include some of the most spectacular and commercially important fish in the world. Although there is a wide range within these two genera, a number of species from each are both euryhaline and anadromous, and, therefore, have very complex life histories. Depending on the species, the young fish spend a variable time in freshwater (weeks to years) before migrating to the ocean to feed and grow into adults. The adult fish then return to freshwater, often traversing thousands of kilometers and overcoming many arduous obstacles, both natural and manmade, to reach their natal spawning grounds.

Coho salmon are representative of trout and salmon species which, as fry and parr, spend between 1-3 years in the freshwater rivers or lakes before migrating to the ocean. The young fish then spend between 1.5 and 3 years in the fertile feeding grounds of the oceans (eg., the northern Pacific for *Oncorhynchus* spp.) before returning to spawn in their natal streams. As is typical of these species, prior to the downstream ocean migration the parr will undergo a complex transformation which serves to preadapt them for existence in a totally foreign milieu: the hyperosmotic environment of the sea. A freshwater lake, compared to the oceans, could be considered as essentially solute-free. Among the vertebrates, perhaps only the amphibians occupy such disparate habitats within their lifetime. If this all important pre-adaptive transition, termed the parr-smolt transformation or smoltification, does not adequately prepare the young fish, then entry into the ocean will lead to osmoregulatory failure and death. The ability for a freshwater fish to alter its metabolism

and physiology to the extent that it may survive in a seawater environment is a remarkable feat in itself. To begin this transition some months before actual ocean entry makes these fish unique.

The focus of my thesis is on this period of transition, the smoltification, of coho salmon, as well as upon the actual entry into the marine environment. In particular, my research is directed at the hormonal regulation of these two stages in the salmon life cycle. The principle regulatory agents in metabolism, physiology and even behaviour are the hormones: compounds secreted by glands into the circulation, having both stimluatory and inhibitory actions on various target tissues. The involvement of hormones in smoltification is not a new idea. Many studies over the last 50 years have shown the importance of several different hormones in the successful transition from parr to smolt. During this time, and particularly in the last 20 years, a great deal of indirect and circumstantial evidence has suggested that one of the hormones involved in the parr-smolt transformation is growth hormone or somatotropin. As the growth-promoting factor, somatotropin has a broad spectrum of activities and numerous target tissues, and many of the parameters which change during smoltification are also parameters which may be influenced by growth hormone. Further, several studies utilizing long-term treatment of parr or pre-smolts with growth hormone preparations (mostly mammalian) have suggested an osmoregulatory role for somatotropin during seawater adaptation. Although smoltification and seawater adaptation may seem to be aspects of the same phenomenon, I feel that they comprise separate yet complementary events within the salmon life cycle.

Research into the involvement of GH in smoltification has been hampered by the neccessity of using mammalian growth hormones, and the lack of a sensitive assay. However, the recent development of specific salmon growth hormone radioimmunoassays

has allowed me to directly investigate whether growth hormone is involved in both the parr-smolt transformation and seawater adaptation in coho salmon. By simultaneously monitoring changes in a number of plasma and tissue parameters, I also attempted to determine some of possible functions or roles of growth hormone in these two events. An understanding of the regulation and coordination of the complex changes which must occur for this transformation to be successful is very important. These fish, and many other members of the Salmoninae, represent a exceedingly unique life history among the animals of the world. Their importance in the ecology of coastal environments cannot be understated, nor can their importance from a economic point of view. However, these animals face increasing pressures, both commercially and environmentally. The rise of aquaculture and salmon enhancement programs in the recent 10-15 years on the West Coast is a reflection of both of these pressures. However, for these programs to be successful, from either point of view, then we must gain a further understanding of how the parr-smolt transformation works, how it may be regulated (internally as well as externally) and how we may be able to use this information to promote successful smoltification and survival in the marine environment.

CHAPTER I

ROLES OF GROWTH HORMONE DURING SMOLTIFICATION

INTRODUCTION

The parr-smolt transformation is achieved via a complex interaction of a number of behavioural, morphological, biochemical and physiological changes, the summation of which is a fish pre-adapted to a marine existence (Fessler and Wagner, 1969;Hoar, 1976;Folmar and Dickhoff, 1980, 1981;Wedemeyer *et al.* , 1980;Gorbman *et al.* , 1982). These transformations appear to be mediated by a number of factors, both from the external environment (eg., photoperiod, temperature, rainfall, and water flow) (Wedemeyer *et al.* , 1980; Brauer, 1982) as well as via internal stimluli. For a number of years, it has been known that many of the changes or transitions which are observed during smoltification seem to be directly and/or indirectly affected by actions of hormones (reviewed by Bern, 1978). Thyroid hormone(s), for example, have been implicated in various aspects of the parr-smolt transformation for some 50 years (Hoar, 1939). However, in the last few years, a great deal of interest in the hormonal regulation of the smoltification process has focussed on the pituitary hormone somatotropin or growth hormone (GH). Olivereau (1954) noted that the number of pituitary somatotroph cells appeared to be greatly increased in Atlantic salmon (*Salmo salar*) smolts, as compared to parr, suggesting a role for GH in the smoltification. Nagahama *et al.* (1977) also noted that the morphology of the growth hormone secreting cells in coho salmon (*Oncorhynchus kisutch*) smolts appeared to suggest increased activity over the parr-smolt transformation. Thus, it seemed that GH may play some role in smoltification.

In the higher vertebrates, particularly in mammals, the actions of GH in increasing somatic growth are well known. This

stimulation of body growth is achieved not only by enhanced cartilage formation and elongation of the long bones, but also via enhanced protein anabolism, reflected by incorporation of amino acids into protein, lowering of circulatory amino acid levels, increased nitrogen retention, and decreased urea formation (Turner and Bagnara, 1976). However, in addition to these direct growth promoting actions in mammals, GH has a number of other metabolic effects. In the blood, circulating levels of glucose increase due to diabetogenic actions. Free fatty acid levels in the blood also increase, due to lipolytic activities of GH. These actions serve to funnel energy-rich compounds from storage depots into the circulatory system, increasing their accessibility for energy-demanding processes, only one of which is growth.

Although the roles of GH are less well established in teleosts, a number of studies have indicated similar actions as seen in mammals. Various GH preparations of a wide range of purity from mammalian and piscine sources have amply demonstrated growth-promoting effects of GH in a number of different fish species (Higgs *et al.*, 1975, 1976, 1977;Clarke, 1976;Clarke *et al.*, 1977;reviewed by Donaldson *et al.*, 1979). GH was also demonstrated to have a protein anabolic effect in the sculpin, *Cottus scorpius*, (Matty, 1962), rainbow trout, *S. irideus*, (Enomoto, 1964), and coho salmon, *Oncorhynchus kisutch*, (Higgs *et al.*, 1975, 1976). Smith and Thorpe (1976) also reported enhanced nitrogen retention following GH treatment of rainbow trout. Indications of free fatty acid mobilisation have been reported in goldfish, *Carassius auratus*, (Minick and Chavin, 1970), coho (Higgs *et al.*, 1975, 1976, 1977;McKeown *et al.*, 1976;Markert *et al.*, 1977) and sockeye salmon, *O. nerka*, (Leatherland *et al.*, 1974;McKeown *et al.*, 1975; Clarke, 1976). Possible diabetogenic effects of GH have also been reported in the sculpin (Matty, 1962), rainbow trout (Enomoto, 1964), sockeye salmon (McKeown *et*

al. , 1975) and coho salmon (McKeown *et al.* , 1976). Thus, there is a solid background of evidence suggesting GH plays an active role in growth and metabolism in teleosts, as it does in mammals and other higher vertebrates.

Many of the various parameters which change during the parr-smolt transformation could potentially be mediated by these actions of GH. Most obvious in this regard is the increased growth rate noted during smoltification (Saunders and Henderson, 1970;Komourdjian *et al.* , 1976:Wedemeyer *et al.* , 1980). Additionally, the parr-smolt transition period is characterised by higher metabolic rates and increased oxygen consumption (Baraduc and Fontaine, 1955;Higgins, 1985), with associated increases in hematocrits (Wedemeyer *et al.* , 1980). These increased metabolic demands are further associated with reductions in muscle and liver glycogen (Fontaine and Hatey, 1950;Malikova, 1959) and body lipid levels (Malikova, 1959;Woo *et al.* , 1978). These depletions in turn are associated with increases in circulatory levels of glucose (Wendt and Saunders, 1973;Woo *et al.* , 1978) and plasma lipids (Woo *et al.* , 1978). The possible importance of GH to the smoltification process was also shown by a number of growth studies done on juvenile salmon. Treatment of parr or pre-smolts with GH not only led to increased growth, but also greatly enhanced survival following seawater entry of Atlantic salmon, *S. salar* (Komourdjian *et al.* , 1976), rainbow trout (Smith, 1956), and sockeye salmon (Clarke *et al.* , 1977), indicating a function of GH in hypoosmoregulation. The parr-smolt transformation in many salmonids also involves increases in gill Na^+,K^+-ATPase enzyme, and the possible role of GH in either the synthesis or induction of the enzyme is an area of active research today. As well, metabolic changes induced by exercise may also involve GH, as is the case in mammals. Circulating GH levels increase in upstream migrating adult sockeye salmon (McKeown and Van Overbeeke, 1972) and in juveniles

forced to swim (McKeown *et al.*, 1975). The role of GH in exercise may be one of increasing the levels of energy-rich compounds in the circulation.

My investigations into the smoltification process in coho salmon are, therefore, based upon the following hypothesis: if GH is involved in the parr-smolt transformation, then plasma levels of GH should increase as smoltification proceeds. Further, it may be possible to ascribe functions to GH by examining correlations with changes in other parameters of smoltification. To examine this hypothesis, I undertook a series of experiments. The first consisted of a weekly sampling study from a population undergoing smoltification. In plasma from these fish, I measured circulating levels of GH as well as levels of glucose, amino acid nitrogen, and free fatty acids. By simultaneously monitoring plasma levels of both GH and the metabolites traditionally affected by GH, I hoped to get a more realistic picture of the dynamics of the actions of GH as well as the interrelationships between various organic compounds. To determine whether GH did affect these plasma parameters, a second experiment was done concurrently with this initial sampling study. In this experiment, plasma metabolite levels were assayed in juvenile coho salmon following injection of GH. This experiment was repeated during several stages of smolt development.

The third experiment was essentially a repeat of the first, but with some important differences. In this study, sampling was initiated at a later date and was continued long after the fish normally migrate to the ocean. In nature, fish which have undergone smoltification and for some reason are prevented from migrating to the ocean will subsequently undergo a desmoltification process. During this desmolting, many of the parameters observed to change during smoltification (such as glycogen and lipid depletions) will revert back to parr-like conditions (Hoar, 1976;Bern, 1978;Folmar and Dickoff, 1980,

1981). By observing this reverse transformation and the corresponding changes in plasma and tissue parameters, including GH levels, I hoped to be able to obtain some further information about the initial parr-smolt transformation. In this second sampling study, in addition to circulating metabolite concentrations, I also assayed tissues to monitor changes in storage levels of these organic compounds. This was done because hormones will often affect turnover rates of metabolites more directly than circulatory levels, and monitoring tissue concentration provides further information on the dynamics of both hormone and metabolite activity. The purpose of these sampling studies, then, was to observe not only whether plasma GH levels increased during smoltification, but also how these changes in GH levels correlated with smoltification-associated changes in both plasma and tissue parameters.

METHODS

Yearling coho salmon, maintained under a natural photoperiod and temperature for Vancouver latitude, were sampled from Capilano Hatchery, North Vancouver on a weekly basis. In 1982, the sampling period was from February 16 to May 25, covering the times when the young fish were obviously parrs, thru smoltification and up to the date of release from the hatchery. In 1983, the sampling regime started April 19 and continued, on a weekly basis, until August 23. The release date for fish from the hatchery in this year was May 31. The subsequent samples (weeks 8 thru 19) were on fish retained in freshwater tanks in the hatchery. These retained fish represent fish which undergo desmoltification. Food was supplied daily *ad libitum* to all fish prior to the release dates, as well as to the fish retained in freshwater in the 1983 sampling year. In both years, the sampling times were always on the same day of the week and at the same times (10:00-11:30 AM).

The sampling procedure was also the same for both years. Fish were randomly netted out from different areas of the raceway, with one or two individuals removed (sampled) from each netting. The caudal peduncle was immediately severed and blood collected in heparinized capillary tubes. In this manner, blood was collected within 20-40s of the fish being netted. This procedure was repeated until 10 fish had been sampled. Care was taken not to unduly disturb the fish in the tanks during the netting procedures. Immediately after collection of the blood, the tubes were placed on ice until centrifuged. Following centrifugation, hematocrits were read and the plasma component was then frozen on dry ice and stored at -20°C until assayed. Weights and lengths of the fish (including the tails) were recorded after the blood sampling procedures were completed. The lengths in all studies were fork lengths. Samples of muscle and liver tissue, collected

in 1983, were also frozen on dry ice and stored at -20° until assayed.

The GH-injection experiments were conducted during the 1982 sampling year. Due to the large amounts of GH required for the injections, and given the limited supply of salmon GH available, this type of experiment was only attempted once. Juvenile coho salmon were obtained from Capilano Hatchery in North Vancouver and held in 120L flow-through tanks under a natural photoperiod. For the purpose of the experiment, 24 fish were removed and placed in three 35L flow-through tanks (8 fish/tank). Their lengths and weights were measured and the fish were grouped such that mean sizes were the same for each treatment group. The fish were allowed to acclimate to the new tanks for 7-8 days. The tanks were then randomly assigned as saline-injected, coho salmon GH-injected (sGH), or ovine GH-injected (oGH, NIH-5-9, 1.0 IU/mg). Teleost saline (0.6% NaCl) was used as the injection medium in all cases.

The test fish were given two successive, daily intraperitoneal injections of oGH, sGH (both 1.0 μg/gm body weight) or saline, 0.5 mL in volume. Fish were sacrificed on the third day of the experiment. Plasma (glucose and AAN) and muscle tissue (AAN and protein) samples were taken as described previously. The first of these injection series was on 27 February, the second on 30 April and the last on 20 June. Injections and samplings were always done between 10:00 a.m. and noon. The fish were not fed during the experiments.

Assay techniques

Plasma GH values were determined by a double-antibody radioimmunoassay, utilizing purified coho or chum salmon GH (Sweeting *et al.*, 1985;Wagner and McKeown, 1986). Analysis of all plasma GH samples were made using triplicates of 20 μL. Assay conditions are as described in Sweeting *et al.* (1985).

Plasma glucose values were assayed by the hexokinase method (SIGMA; kit #15-UV). Triplicate determinations were done for most samples, utilizing 5 μL of plasma. Plasma amino acid nitrogen was assayed by a modified method of Rosen (1957). Twenty μL of plasma were added to 380 μL of 5% TCA. This was allowed to sit on ice for 1 hour and then spun in an IEC DR-60 centrifuge for 10 minutes at 4000g. Three 100 μL aliquots of the supernatant were then placed into 12x75mm test tubes. To each tube was added 50 μL acetate-cyanide (0.0002M NaCN in acetate buffer, pH 5.35). This reagent decomposes rapidly and was therefore made up fresh each day. To this mixture, 50 μL of ninhydrin reagent (3.0 gm in 100 mL 2-methoxyethanol)(Mallinckrodt Chemicals) was added. The tubes were placed in a water bath at 100°C for 15 minutes. Immediately after removal from the water bath, 2.5 mL of diluent (isopropyl alcohol:distilled water, 1:1) was vigorously added and the mixture allowed to cool to room temperature. The samples were read at 570 nm on a Perkin Elmer Model 55 Spectrophotometer. Plasma values were determined against a standard curve utilizing 1.0 - 15.0 mg% glycine. One mg of glycine was determined to be equivalent to 0.186 μg nitrogen.

Plasma free fatty acid levels were determined using a Waters High Pressure Liquid Chromatography (HPLC) Modular System. The protocol was modified from the procedure of Borch (1975), as follows: 25 μL of plasma were added to 1.0 mL microcentrifuge tubes. Approximately 4.5 μL 1N HCl was added to bring the pH down to 1.5. NaCl was added to saturation, followed by 250 μL of ethylene glycol to extract the lipid fraction. After 15 minutes, the tubes were centrifuged for 3 minutes at 15000g (Eppendorf, Model no. 5414). This resulted in an upper layer of ethylene glycol (containing the lipid fraction), separated from a lower aqueous phase by a thin disk of proteinaceous material. To prevent disturbance of the interface, only 200 μL of the upper layer was removed to a 12X75mm test tube. Another 250 μL of

ethylene glycol was added, the sample was allowed to sit for 5 minutes, and recentrifuged. A 250 μL aliquot was removed, and added to the first 200 μL aliquot. This last step was repeated one more time, resulting in 700 μL of lipid-containing solvent in the 12x75 test tube. This extractant was evaporated to dryness under nitrogen and then placed under vacuum for 20-40 minutes to ensure total evaporation. The free fatty acids in each tube were esterified to 4-bromophenacyl bromide (Sigma)(250 μg in 500 μL of HPLC-grade acetonitrile (BDH Chemicals) with N,N-diisopropylethylamine (Sigma)(1.0 μL/tube) as a catalyst. The samples were then capped and heated in a water bath at 55°C for 20 minutes. The tubes were subsequently recapped and kept at room temperature until assayed, usually the same day. Fatty acid standards (Sigma) were esterified in the same manner. Separation of the FFA:4-bromophenacyl bromide esters was obtained on a reverse-phase C18 column (25 X 4 mm I.D.), 5 μm bead (Waters, Toronto), operated at 40°C. The mobile phase was initially 60% acetonitrile (in water; pH adjusted to 3.10 with H_3PO_4) for 5 minutes, followed by a linear gradient to 100% acetonitrile in 80 minutes at a flow rate of 1.0 mL/minute. The esters were detected on a Waters spectrophotometer (model no. 481) at a wavelength of 254 nm. The peak areas and retention times of 40 μL aliquots of the samples were integrated and quantified by an inline computer system (Waters Model 840 Data and Chromatography Control Station). Table 1 shows the scientific and common names of the 18 free fatty acids analysed in these studies.

Liver and muscle glycogen levels were determined by the following procedure: pre-weighed amounts of wet tissue were homogenized with a glass pestle in a volume of citrate buffer (0.1M citric acid, 0.1M sodium citrate, 2.5 g/L sodium flouride; pH 4.2), resulting in 40 mg muscle/mL or 10 mg liver/mL. Tissues were homogenized using a Black and Decker variable speed drill, 15 strokes for liver samples and 35 strokes for muscle samples.

Table 1: Nomenclature and Bond Positions of the Fatty Acids
Fatty acids are generally described by using two numbers separated by a colon. The first identifies the number of carbon atoms and the second number designates the number of double bonds. Thus, 18:3 would designate a fatty acid containing eighteen carbons and three double bonds. The position of the double bond(s) is commonly described by omega (ω) or by 'n', indicating the position of the first double bond counting from the methyl carbon end of the acid.

Common name	Systematic name	Abbreviation
Lauric acid	Dodecanoic acid	12:0
Tridecanoic acid	Tridecanoic acid	13:0
Myristic acid	Tetradecanoic acid	14:0
Myristoleic acid	9-tetradecanoic acid	14:1
Pentadecanoic acid	Pentadecanoic acid	15:0
Palmitic acid	Hexadecanoic acid	16:0
Palmitoleic acid	*cis*-9-hexadecenoic acid	16:1 (n-7)
Palmitelaidic acid	*trans*-9-hexadecenoic acid	16:1 (n-7)
Margaric acid	Heptadecanoic acid	17:0
Stearic acid	Octadecanoic acid	18:0
Oleic acid	9-octadecenoic acid	18:1 (n-9)
Linoleic acid	9,12-octadecadienoic acid	18:2 (n-6)
α-Linolenic acid	9,12,15-octadecatrienoic acid	18:3 (n-3)
Arachidic acid	Eicosanoic acid	20:0
Eicosadienoic acid	11,14-eicosadienoic acid	20:2 (n-6)
Eicosatrienoic acid	11,14,17-eicosatrienoic acid	20:3 (n-3)
Arachidonic acid	5,8,11,14-eicosatetraenoic acid	20:4 (n-6)
Timnodonic acid	5,8,11,14,17-eicosapentaenoic acid	20:5 (n-3)
Behenic acid	Docosanoic acid	22:0
Cervonic acid	4,7,10,13,16,19-Docosahexaenoic acid	22:6 (n-3)

The crude extracts were centrifuged for 12 minutes at 3000g. A one mL aliquot of the supernatant was then added to a 12x75 mm test tube containing 1.0 mg amyloglucosidase (SIGMA; EC 3.2.1.3.). The tube was capped and heated in a water bath at 55°C for 20 minutes. The tubes were then vortexed and recentrifuged at 3000g for 5 minutes. The supernatant was then analyzed for glucose by the hexokinase method (SIGMA #15UV). Samples were read against glycogen standards treated in the same manner. An aliquot of supernatant from the first spin was also analyzed to determine initial or free glucose levels. Tissue glycogen levels were obtained by subtracting the initial free glucose levels from the total (initial + glycogen-derived) glucose levels.

Muscle protein was determined using the Bio-Rad Protein Assay (Cat #500-0006), based on Bradfords' method (1976). Briefly, the protocol was as follows: 100 mg (wet weight) of muscle was removed from the left dorsal quadrant of the fish, taking care to avoid the lateral red musculature. This sample was placed in a 12x75 mm test tube with 1.0 mL 2N NaOH and capped with tinfoil. The mixture was placed in a water bath at 100°C for one hour to dissociate the tissue. Immediately following this bath, 1.0 mL of 10% trichloroacetic acid (TCA) was added to the tube to precipitate the protein. The mixture was vortexed and spun for 8.0 minutes at 3200g in an IEC Model DR-60 centrifuge. Three aliquots of 100 µL were removed from the supernatant for muscle amino acid nitrogen determination, using the assay previously discussed. The remaining supernatant was discarded and the pellet resuspended in 1.0 mL of distilled water. From this suspension, 50 µL was added to 2.5 mL of dye reagent (1:40 distilled water). The samples were read at 595 nm on a Perkin Elmer Model 55 Spectrophotometer against a standard curve of albumin, treated in the same manner as the samples.

Statistics

Results from the weekly sampling experiments were analyzed for differences by oneway analyses of variance (ANOVA), using Student Newman-Keuls multiple ranges test. All differences discussed in the results are significant at the 0.05 level, unless specific P values are shown. Correlations were done with Pearsons correlation matrix (SPSSX), using .01 as the significance level.

The results derived from the injection experiments were analysed for differences utilizing ANOVA, with Student Newman-Keuls multiple range test at the 0.05 significance level. For the FFA analyses from the injection experiments, a T-test was utilized to examine differences as there were no results from the second injection series. Again, all differences discussed are significant ($P < .05$).

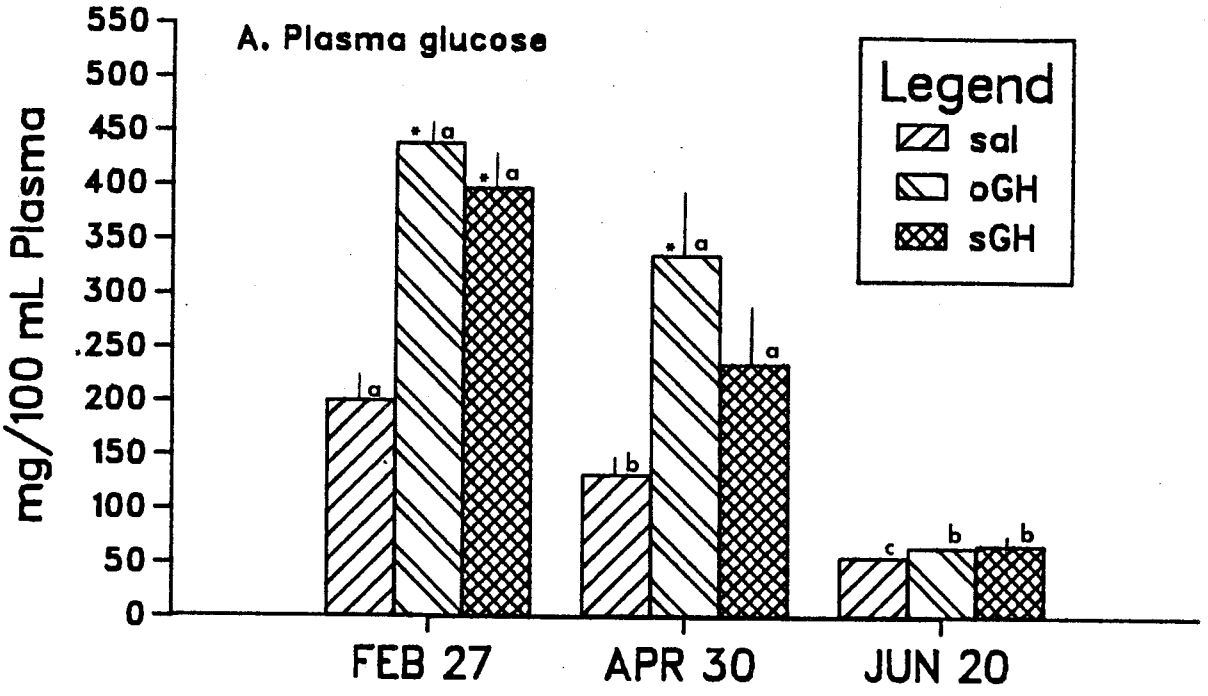
RESULTS

INJECTION EXPERIMENTS

Both the sGH- and the oGH-treated fish exhibited significantly elevated plasma glucose levels (Figure 1a) in the initial injection experiment. Only oGH was effective in the April 30 injection, although the sGH-treated fish did demonstrate a trend towards higher levels. Neither hormone elicited a response in the third injection (June 20). At no time were there any differences in plasma glucose between the two growth hormone-treated groups. Plasma AAN values (Figure 1b) were not different from the saline controls in any injection experiment. Plasma from the saline-injected controls in the third experiment would not separate upon centrifugation except in one sample, thus there was an 'n' of only one in this group. Plasma glucose was not affected by this problem.

Both glucose and AAN concentrations in the plasma decreased over the three injection dates. Plasma glucose levels in all 3 treatment groups were lower in the June 20 injections than in either the February 23 or April 30 injections (Figure 1a). Circulating plasma glucose in the saline-treated fish of the April 30 injection experiment was also lower than the level seen in the February 23 saline-injected fish, whereas neither of the GH treated groups had different plasma glucose titers between the first and second injection experiments. Plasma AAN also demonstrated steadily declined over the 3 injection dates (Fig. 1b), although the results were not as dramatic as observed with plasma glucose. Saline controls had lower pAAN in April 30 than they did in February 23. While both ovine and salmon GH- treated groups in the June 20 injections possessed lower pAAN levels than observed in the February 23 groups, only the oGH-treated fish were significantly lower in pAAN than the corresponding April 30 group.

Figure 1: Plasma glucose (A) and amino acid nitrogen (B) levels in juvenile coho salmon injected with saline, ovine GH or salmon GH. Each histogram represents a mean (n=8) ± S.E.M. Stars indicate values significantly ($P < .05$) different than controls (saline injected). Within group differences are indicated by letters, such that means with the same letters are not significantly different.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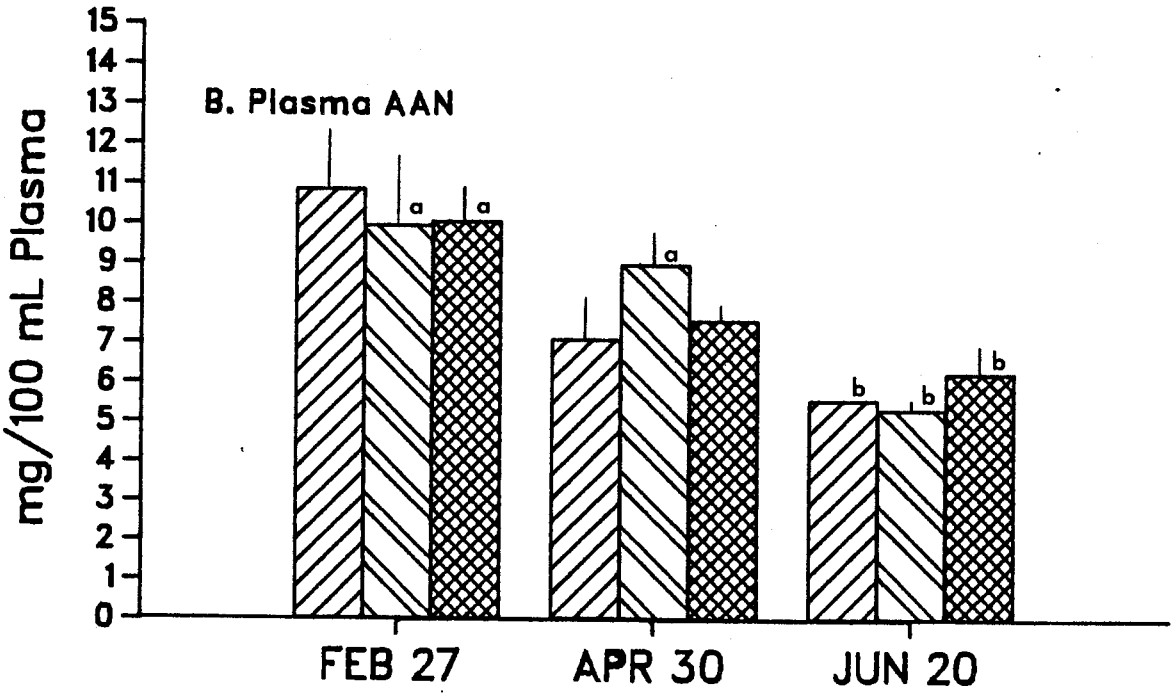
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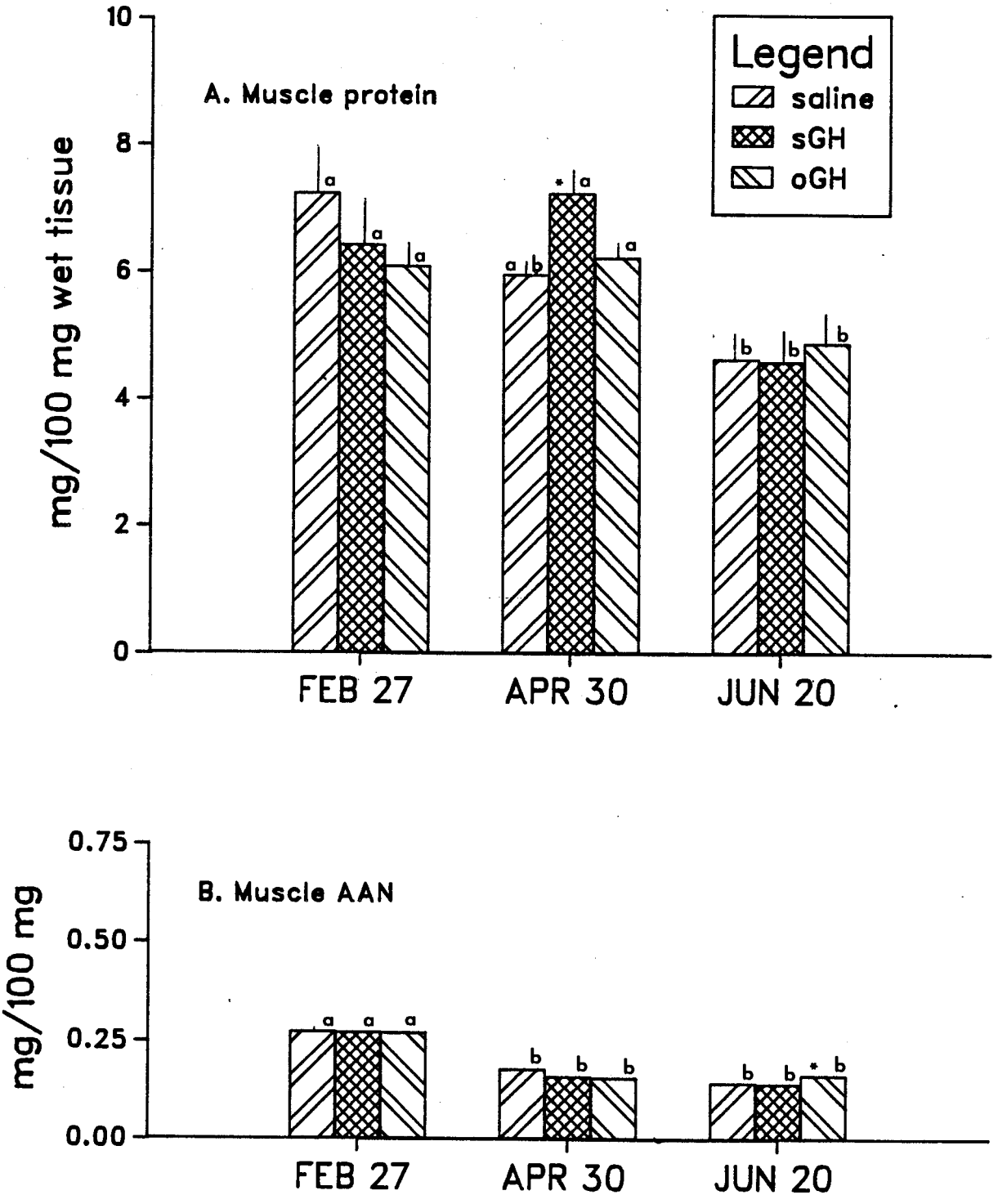
APR 30
JUN 20

As seen in the plasma AAN titers, there were no effects of either sGH or oGH on muscle AAN levels in the first or second injection experiments (Figure 2b). In the third injection (June 20), the oGH-treated fish had significantly higher muscle AAN levels than either the saline-injected controls or the sGH-treated fish. Muscle protein (Figure 2a) was also not affected by GH treatment in the first injection experiment. In the second injection experiment, however, sGH-treated fish possessed higher muscle protein content than either the saline- or the oGH-injected groups. In the third injection, there were once again no differences between the three groups. As in the plasma levels of glucose and AAN, muscle content of AAN and protein declined in all three treatment groups over the entire experimenal period (ie, from February to June). Muscle AAN concentrations in both the second (April 30) and third (June 20) injections were significantly lower than seen in the first experiment(February 23). Similarly, muscle protein in the third injection was lower in all three treatments than in the initial injection groups. These levels were also lower than seen in the second injection in the sGH- and oGH-treated groups, but not in the saline-injected controls (Fig. 2b).

Plasma free fatty acids were analyzed on the plasma remaining after the glucose and AAN assays. It was not possible to make any analyses of the April 30 injection experiment, due to a lack of plasma. Additionally, the unseparated blood from the June 20 controls proved unsuitable for FFA analysis. Lastly, results of the oGH-treated fish from the February 23 injection were not usable due to a contaminant in the assay reagents. With these restrictions in mind, the data must be considered to be preliminary. Although the data is not shown, some tentative conclusions can be drawn. First, in the February injection experiment, the sGH was ineffective in significantly increasing plasma FFA levels over the saline controls, although the

Figure 2:Muscle protein (A) and amino acid nitrogen (B) concentrations in juvenile coho salmon injected with saline, ovine GH or salmon GH. Each histogram represents a mean (n=8) ± S.E.M. All values are plotted in mg/100 mg wet tissue. S.E.M. Stars indicate values significantly ($P < .05$) different than controls (saline injected). Within group differences are indicated by letters, such that means with the same letters are not significantly different.

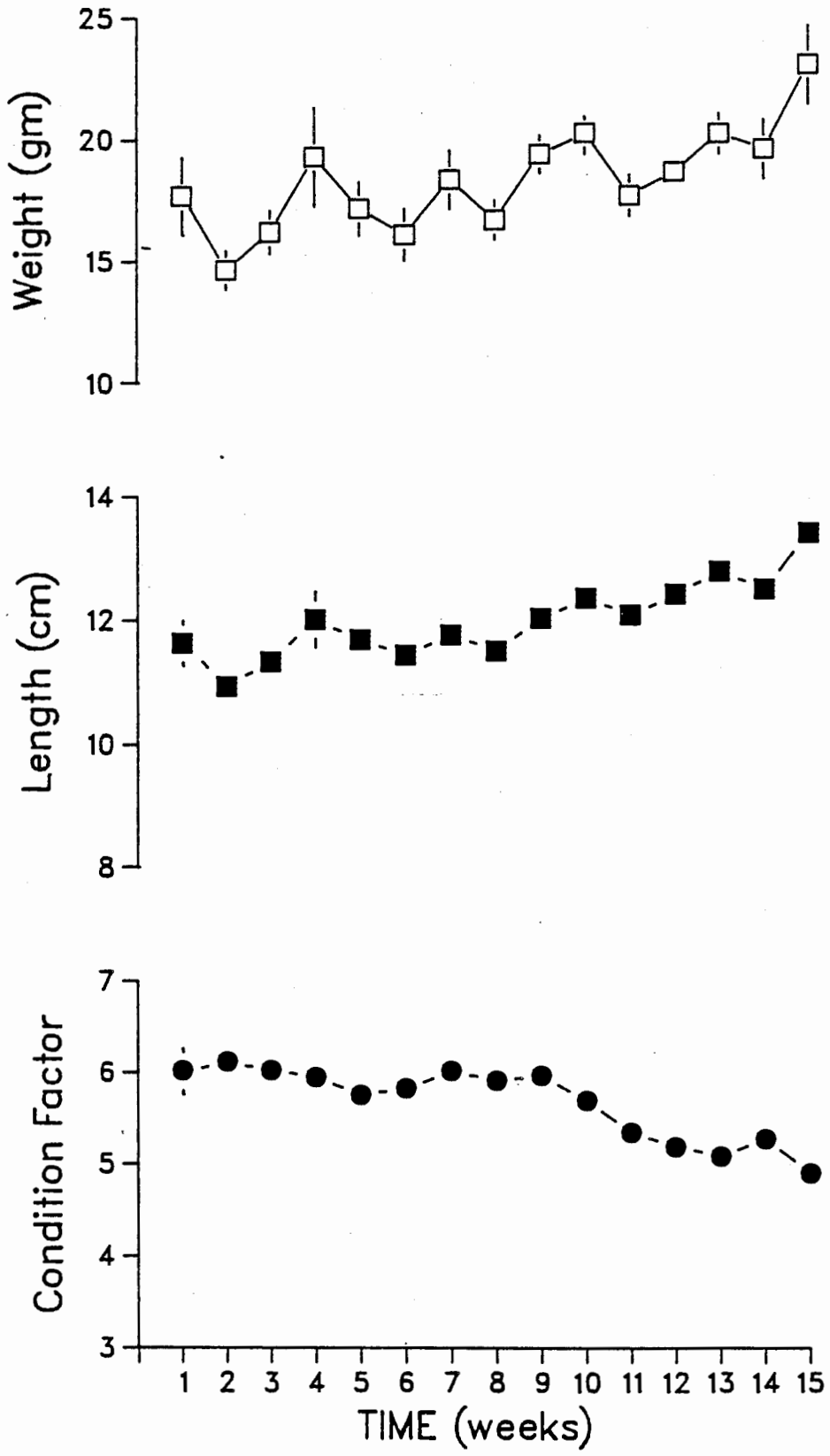

A. Muscle protein
mg/100 mg wet tissue
10
8
6
4
2
0
Legend
saline
sGH
oGH
FEB 27
APR 30
JUN 20
B. Muscle AAN
mg/100 mg
0.75
0.50
0.25
0.00
FEB 27
APR 30
JUN 20

concentration of most of the individual FFAs did tend to be higher in plasma of the sGH-treated fish. Second, the plasma levels of FFA in the groups injected in June were lower than the levels seen in February, in agreement with the overall declines seen in plasma glucose and AAN titers. Third, there was no difference in plasma FFA levels (individual or totals) between sGH- or oGH-treated fish in the June 20 experiment. This was also seen with respect to glucose and AAN in the June fish, but the lack of a saline control for FFA makes it difficult to draw conclusions from this result.

1982 WEEKLY SAMPLING

The morphological data from the 1982 sampling year is shown in Figure 3. Weight of the fish (Fig 3a) increased rapidly over the summer, the week 15 group being significantly larger than weeks 1, 2, 3, 5, 6, 8 and 11. Figure 3b displays the length data for this sampling period. As seen in the weight data, fish from week 2 were among the smallest sampled, being shorter than fish sampled from weeks 4, 9, 10, 11, 12, 13, 14, and 15. Indeed, fish from week 15 demonstrated the largest mean length, being longer than fish sampled from every previous week with the exception of those from week 13. The condition factor,or growth index, of these fish is shown in Figure 3c (condition factor = $weight/length^{3.25}$). While there was a general decline in condition factor over the entire 15 weeks of sampling, after week 10 there seemed to be an increase in the rate, such that the condition factor from weeks 11-15 were all lower than that seen from weeks 1-10. Regression analyses over time demonstrated the general increases in weight ($Y = .3468X + 15.579; R^2 = .1386$) and length ($Y = .1242X + 11.001; R^2 = .3193$), both significant ($P < .001$). Condition factor, on the other hand, exhibited a negative regression ($Y = -.0789X + 6.2977; R^2 = .4428$), also highly significant ($P < .001$).

Figure 3:1982 coho smoltification study: morphological data including weight (open squares), length (closed squares) and condition factor (see text for explanation) (closed circles). All points represents means (n=10) ± S.E.M. See text for detailed discussion.

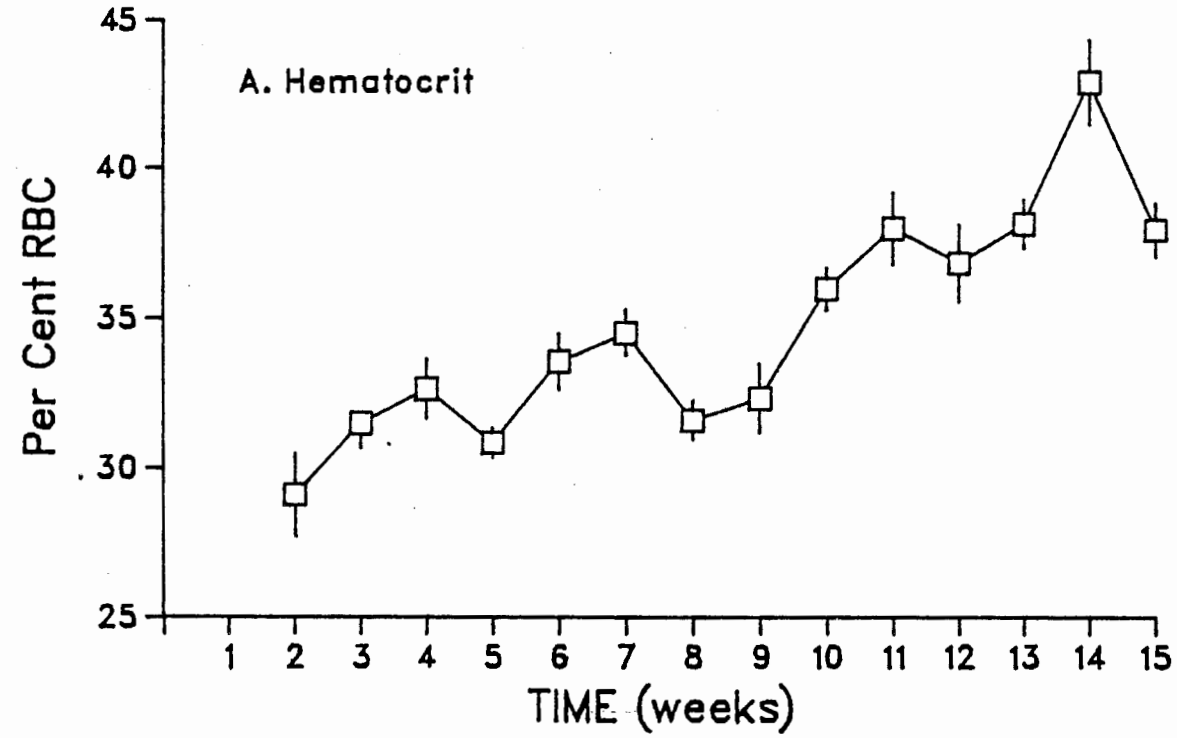

Weight (gm)
25
20
15
10
Length (cm)
14
12
10
8
Condition Factor
7
6
5
4
3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)

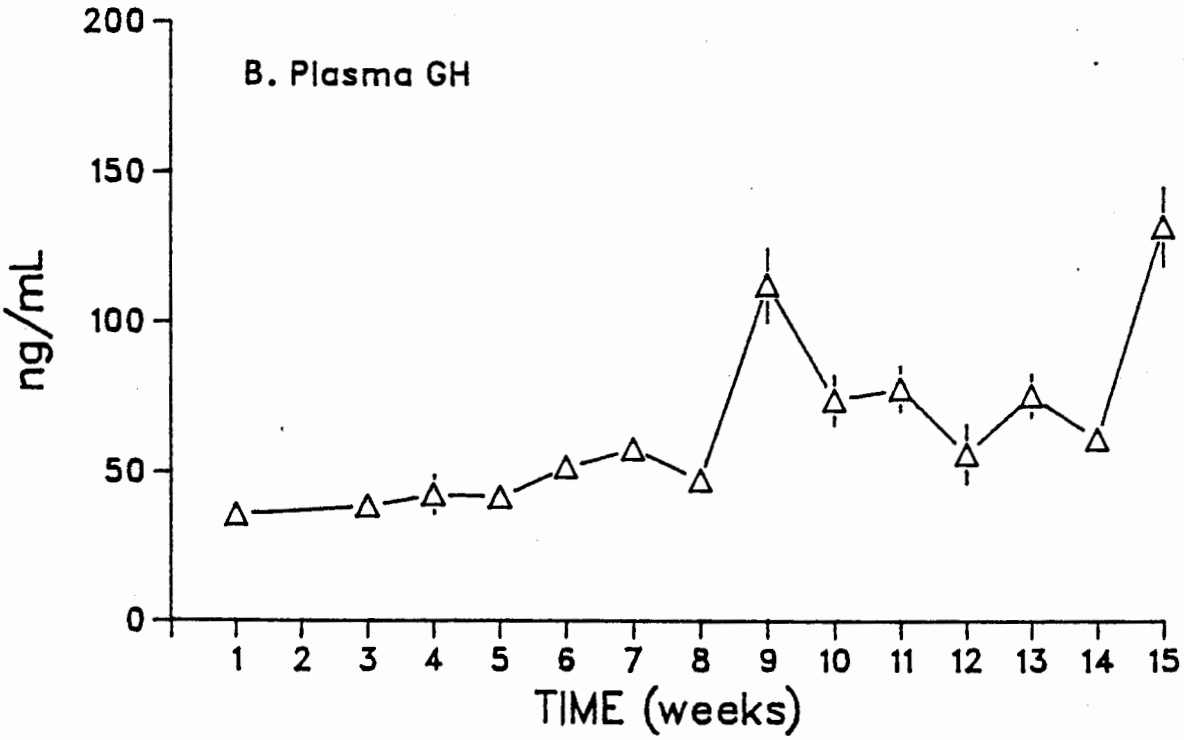
Over the 15 weeks of sampling, the hematocrits of the smolts generally increased (Fig. 4a). Data for this parameter was not collected for the first week. The highest level of hematocrit was reached on week 14 (42.92%) and this value was higher than all other weeks. Regression analysis showed a highly significant ($P < .001$), positive slope for hematocrit over time ($R^2=.456; Y = .789X + 27.988$). Plasma GH levels, shown in Fig. 4b, exhibited variable but generally increasing values over the 15 weeks of sampling (note:GH values unavailable from week 2). Fish from weeks 9 and 15 had higher plasma GH titers than all other weeks. As well, the GH concentration of fish from week 11 were greater than those in fish from weeks 1, 3, 4 and 5 while the levels of GH in the plasma of fish sampled in weeks 10 and 13 were higher than those levels from weeks 1, 3 and 5. As would be expected from the data, regression analysis again showed a highly significant ($P < .001$) increase over the 15 weeks ($R^2=.285; Y = 4.62X + 26.44$).

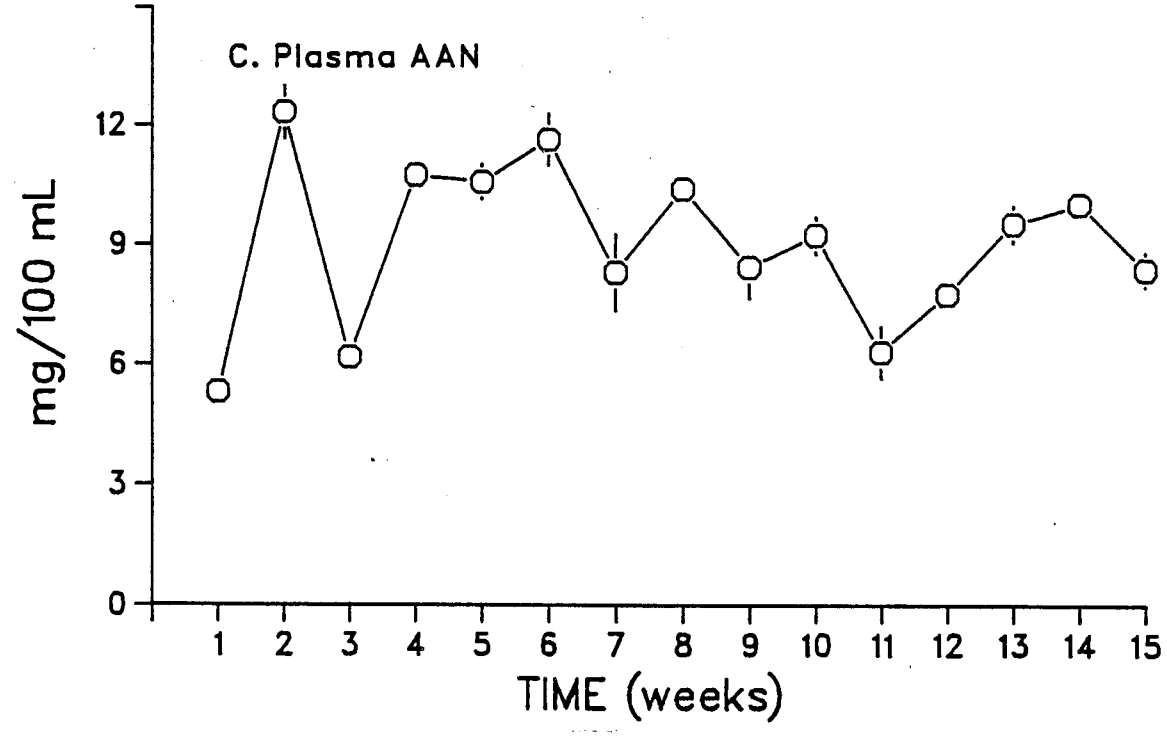
Plasma AAN levels, on the other hand, were generally stable over the 15 weeks of sampling (Fig. 4c). Regression analysis of this parameter showed no significant change with time ($R^2=.001; P=.344$). However, within the 15 weeks, there were some significant differences such that fish from weeks 1, 3 and 11 had plasma AAN levels lower than measured in all other weeks. Plasma glucose titers, shown in Figure 4e, also showed no real change over the sampling period ($R^2=.0139; P=.0767$). However, there were some peak values seen over the 15 weeks, such that the plasma glucose level at week 12 was higher than the levels seen in fish from weeks 1, 2, 6, 9 and 15. Plasma glucose concentrations from week 11 were also higher than from weeks 6, 9 and 15..

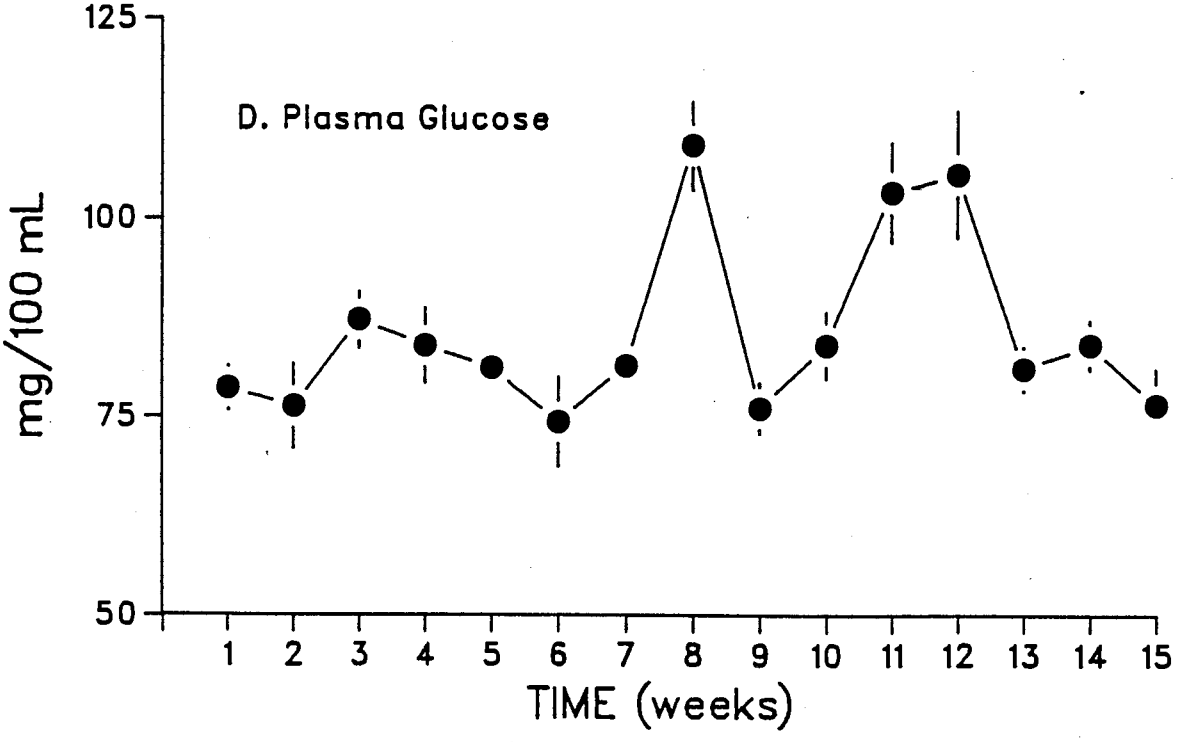
Figures 5-7 show the plasma FFA profiles for the 1982 sampling year. The relatively small size of the fish limited the total amount of plasma collected. As such, the n sizes of these FFA profiles are less than the 9 or 10 individuals making up the means of the previous parameters. The plasma levels shown for

Figure 4:1982 coho smoltification study: plasma hematocrits (A), GH levels (B), amino acid nitrogen levels (C), and glucose levels (D). Each point represents the mean (n=8-10) ± S.E.M. Hematocrits are expressed as percent, GH values are expressed as ng GH/ml plasma, and both amino acid nitrogen and glucose are expressed as mg/100 ml plasma. See text for detailed discussion.


A. Hematocrit
Per Cent RBC
45
40
35
30
25
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)


B. Plasma GH
ng/mL
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)


C. Plasma AAN
mg/100 mL
0
3
6
9
12
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)


D. Plasma Glucose
mg/100 mL
50
75
100
125
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)

weeks 7, 8 and 11 consists of pooled plasma from 6 to 8 of the weekly total. Thus, although the sample size for these weeks is only 1, it represents a mean of several fish from that week. Additionally, there was no plasma available from the initial 2 weeks of sampling in this 1982 experiment, so the plasma profiles of FFA begin with week 3 fish, which represent fish sampled March 2, 1982.

The total saturated free fatty acid concentration in the plasma of this weekly sampling study is shown in Figure 5d, with the 9 individual saturated FFAs plotted in Figs 5a-c. There was a large increase in total satFFA following the week 7 sample, such that values measured in weeks 9, 10, and 12-15 were higher than seen in weeks 3-7. Further, the week 12 value was higher than values obtained in other weeks except week 11. The regression analysis for the total satFFA over the 12 weeks ($Y = 26.09X + 148.51$) was highly significant ($R^2 = .555; P < .001$).

As can be seen in Figs. 5a-c, the large increase in total satFFA was due primarily to large increases in C16:0 and C18:0 [1] although most of the saturated FFAs show increases over weeks 7-9, with the exception of C17:0 (heptadecanoic acid) which decreased over the 12 weeks. The weekly plasma concentrations of the saturated fatty acids lauric (C12:0), tridecanoic (C13:0) and myristic (C14:0) acid are shown Figure 5a. The high level of lauric acid seen in the plasma of fish sampled in week 8 was higher than all other weeks, except fish from weeks 11 and 12. The level of lauric acid seen in week 12 fish was higher than that in weeks 4-7, 10, 13 and 15. Plasma tridecanoic acid exhibited only 1 peak value, seen in the week 10 fish, which was higher than levels seen in weeks 6, 12, 13, 14 and 15. Plasma myristic acid showed several small peaks, but generally, the levels in weeks 10 to 15 were higher than those measured in weeks

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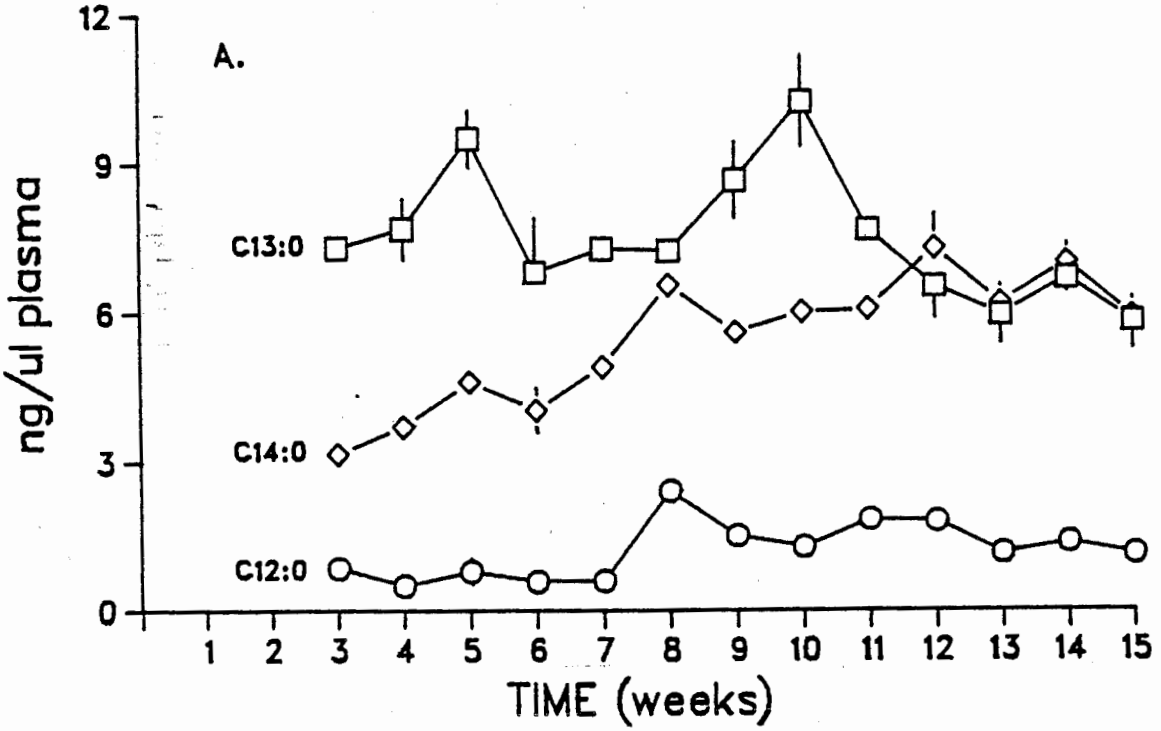
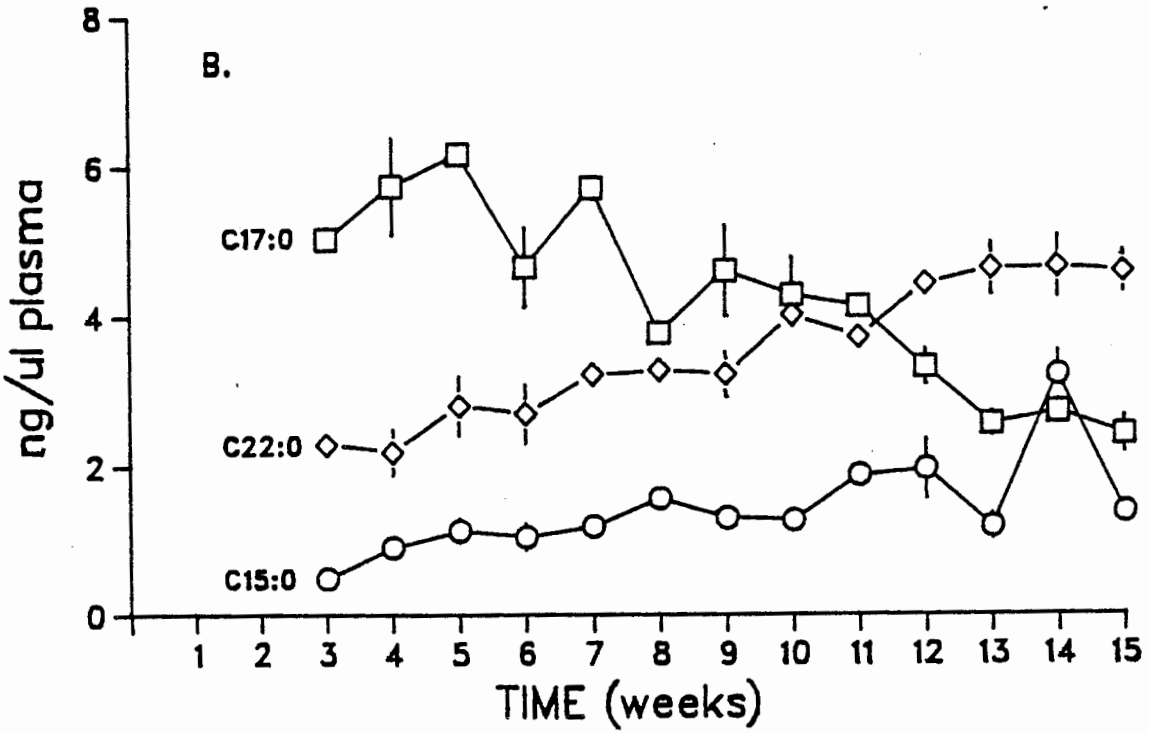
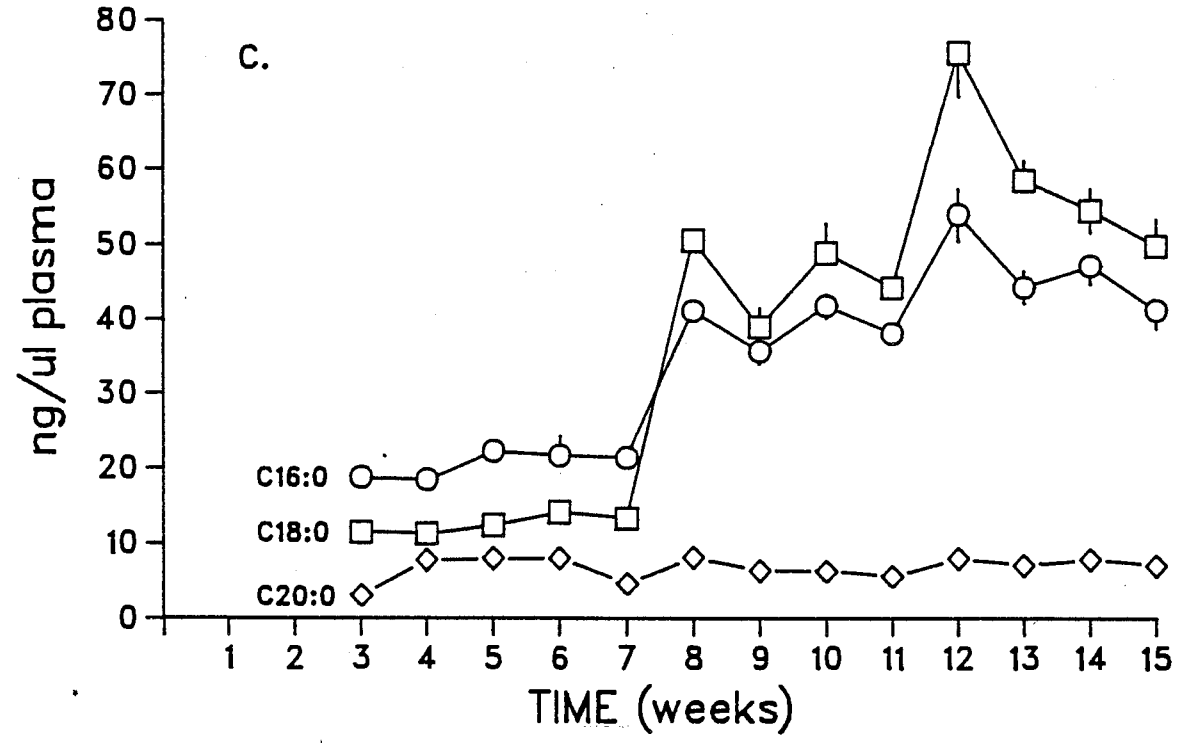
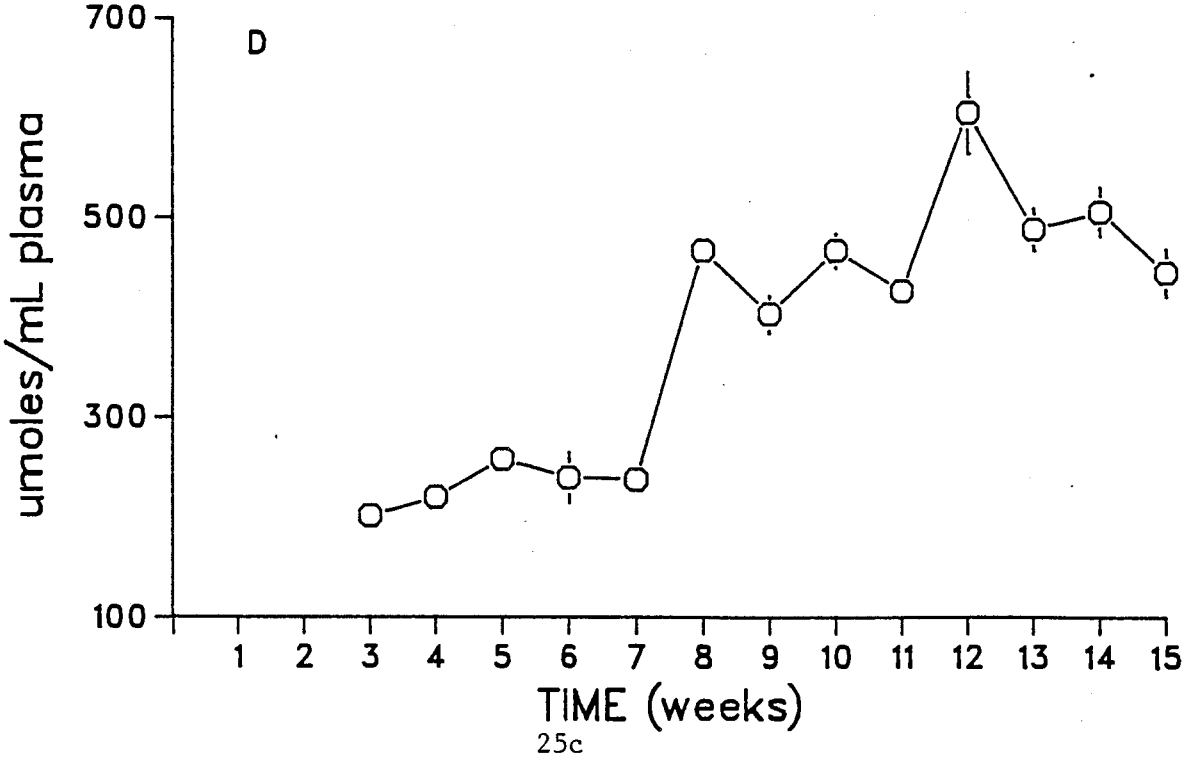
[1]See table 1 for detailed descriptions of fatty acid nomenclature.

Figure 5:1982 coho smoltification study: plasma saturated free fatty acid levels. (A) Lauric acid (open circles), tridecanoic acid (open squares) and myristic acid (open diamonds). (B) Pentadecanoic acid (open circles), heptadecanoic acid (open squares) and arachidic acid (open diamonds). (C) Palmitic acid (open circles), stearic acid (open squares) and behenic acid (open diamonds). (D) Total saturated free fatty acids. Carbon numbers are shown for each acid on the appropriate curve. Each point represents the mean (n=8-10) ± S.E.M. Plasma values for A, B, and C are in ng FFA/μl plasma whereas D is expressed as μmoles FFA/ml plasma. See text for detailed discussion.


A.
C13:0
C14:0
C12:0
ng/ul plasma
TIME (weeks)
0
3
6
9
12
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15


B.
C17:0
C22:0
C15:0
ng/ul plasma
TIME (weeks)
0
2
4
6
8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15


C.
ng/ul plasma
80
70
60
50
40
30
20
10
0
C16:0
C18:0
C20:0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)


D
umoles/mL plasma
700
500
300
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)

3, 4, and 6.

Figure 5b displays the weekly plasma profiles of pentadecanoic (C15:0), heptadecanoic (C17:0) and arachidic (C20:0) acids. Levels of pentadecanoic acid remained low over much of the sampling period, with the peak value seen in week 14 fish being higher than all other weeks. As mentioned, plasma levels of heptadecanoic acid generally decline over the 15 weeks of sampling, such that fish sampled in weeks 4 and 5 displayed higher concentrations than observed in fish from weeks 10, 12, 13, 14 and 15. Further, fish sampled in weeks 6, 9 and 10 had higher plasma titers of heptadecanoic acid than fish sampled in the last three weeks prior to release from the hatchery (ie., weeks 13, 14 and 15). Arachidic acid showed a well defined increase in plasma concentration over the 15 sampling weeks, such that the levels of this fatty acid seen in plasma samples obtained in week 12 to 15 were higher than the levels observed in fish from weeks 4, 5 and 6.

Plasma levels of the saturated fatty acids C16:0, C18:0 and C22:0 are shown in Fig. 5c. Despite the wide ranges seen in the concentrations of behenic acid (C22:0), there were no significant differences seen over the sampling period. Palmitic acid (C16:0) and stearic acid (C18:0) made up the large proportion of both the saturated fatty acids and the total free fatty acids in the plasma of these fish. Both of these compounds exhibited large increases during the 15 weeks. Fish from week 12 displayed the highest plasma concentrations of palmitic acid, being greater than the levels observed in fish from weeks 3-7, 9, 10, 13 and 15. Week 14 fish also had higher titers of palmitic acid in the plasma than fish sampled in weeks 3-7 and 9. Further, the mean plasma titers of palmitic acid seen in fish from weeks 10, 13 and 15 were higher than seen in fish obtained in weeks 3-7. Plasma stearic acid levels in fish sampled in weeks 3, 4, 5, 6 and 7 were all lower than the values for this fatty acid seen in weeks

8-15.

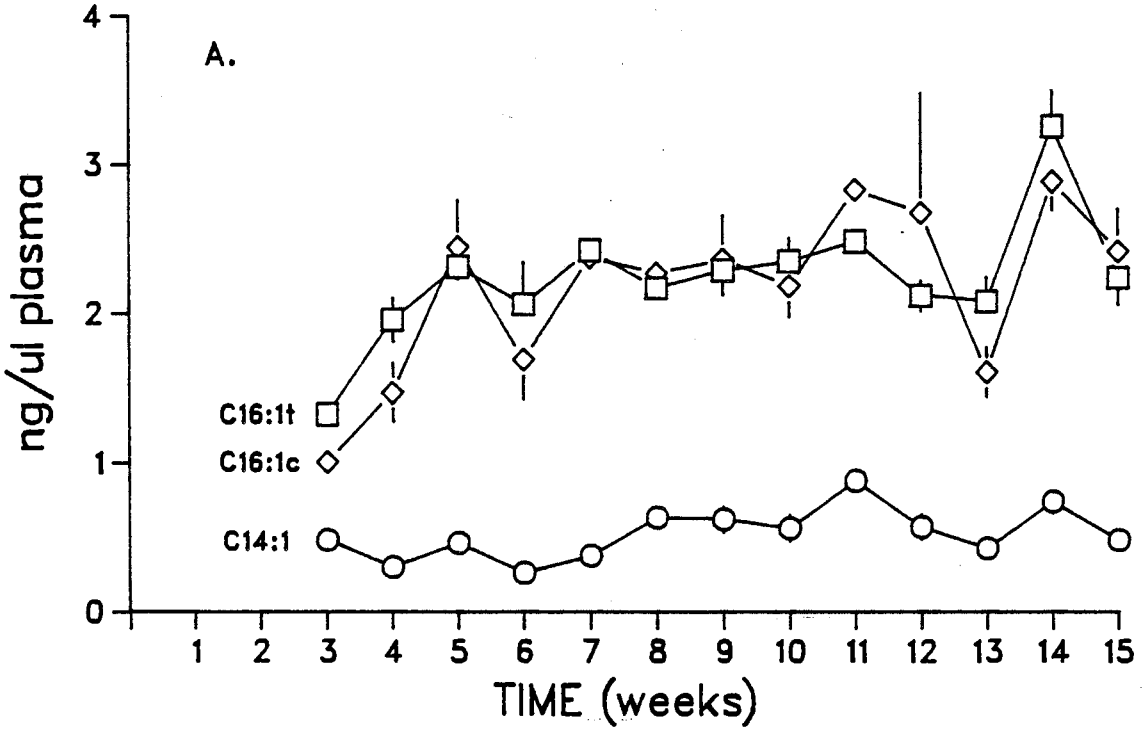
Levels of unsaturated free fatty acids (unsatFFA) in the plasma are shown in Fig. 6a-c, with the total unsatFFA displayed in Fig. 6d. Unlike their saturated counterparts, the unsatFFA did not display large increases although many did show a peak value in week 14. This value, for the total unsatFFA, was higher than all other values except those seen in weeks 7, 8 and 11. Again, the lack of significance between these values may be due to the single pooled samples. The regression of total unsatFFAs was again positive ($Y = 2.779X + 105.83$), although the significance was not as strong ($R^2 = .100; P = .012$).

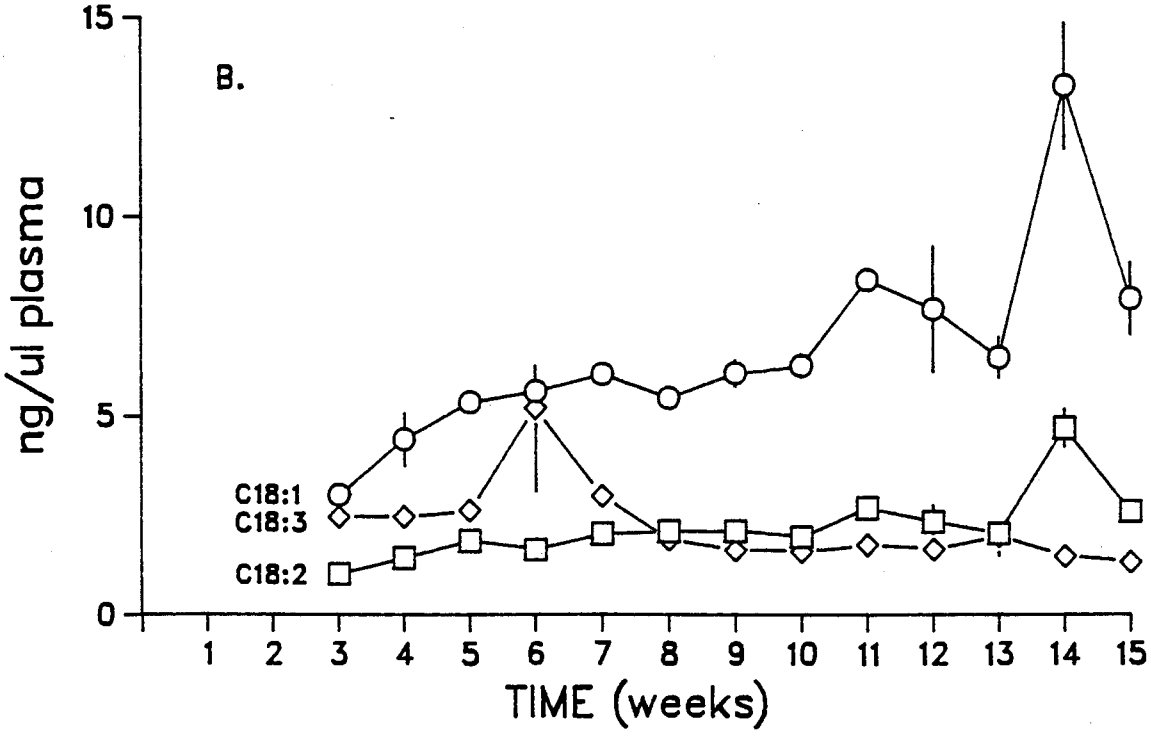
Myristoleic (C14:1) and the two palmitic monounsaturates (C16:1c and C16:1t) are profiled in Figure 6a. The week 14 value for myristoleic acid was higher than the levels seen in weeks 4, 6 and 13 whereas for palmitoleic acid, the week 14 value was higher than those measured in weeks 3-6, 9, 10, 12, 13 and 15. There were no differences in plasma palmiteleic acid (C16:1c) over the 15 weeks.

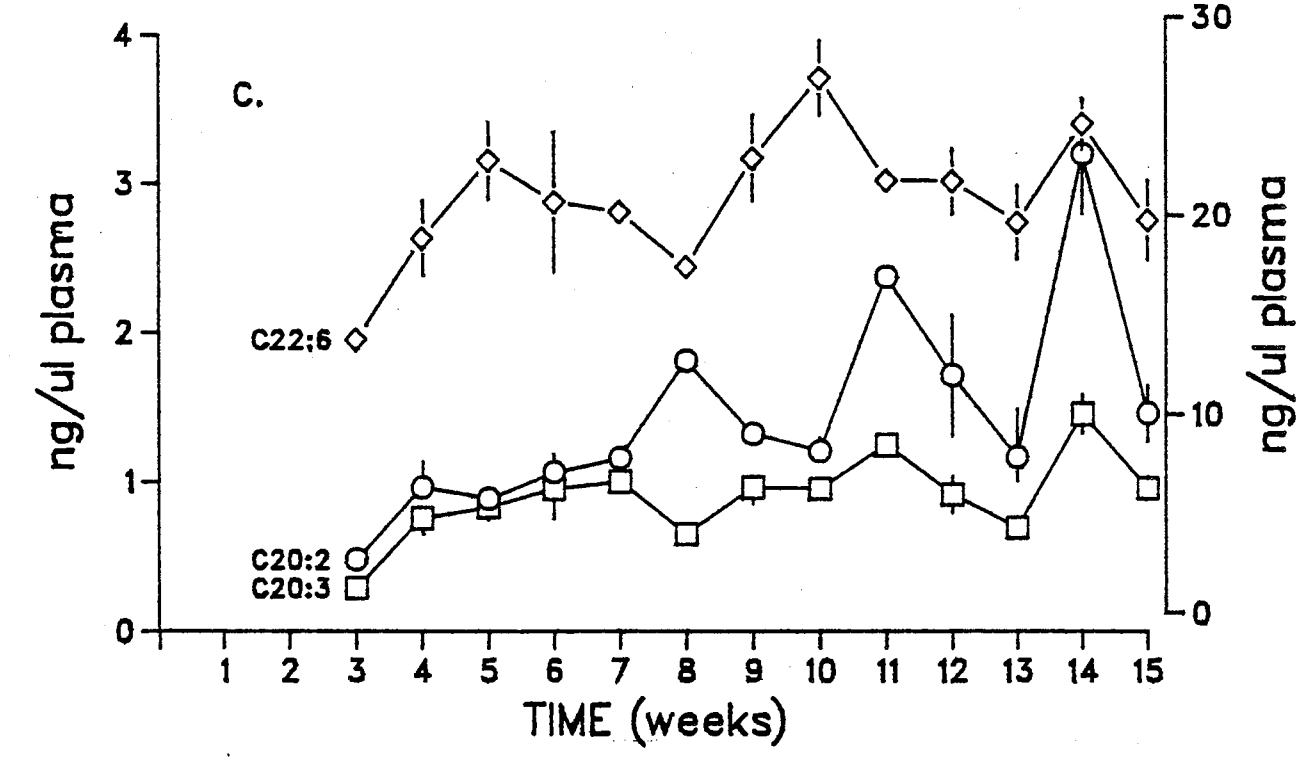
The unsaturated fatty acids of the 18 carbon group are shown in Fig. 6b. As seen in the previous unsatFFA, there was a peak plasma level seen in week 14 fish. For oleic acid (C18:1) this value was higher than all other weeks, except weeks 7 and 8 (probably due to the 'single' sample size rather than to a lack of differences between the means). The week 14 peak in linoleic acid (C18:2) levels was higher than all other weeks. Week 14 fish did not display elevated plasma titers of linolenic (C18:3) acid. The highest plasma level of this unsatFFA was seen in the week 6 fish, which was significantly greater than the levels seen in fish obtained in weeks 5, 9, 10 and 12-15.

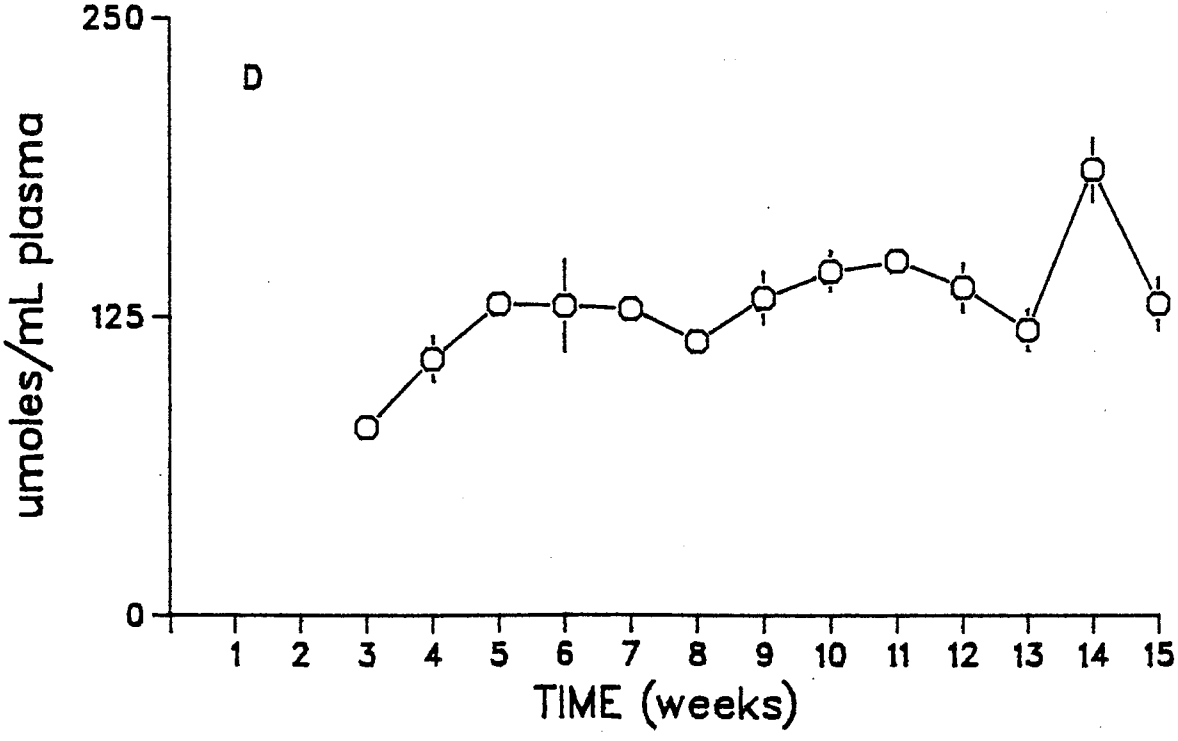
The last three unsatFFA monitored are displayed in Fig. 6c. Plasma concentrations of eicosadienoic acid (C20:2) again showed a distinctive peak in the fish sampled in week 14. This value was

Figure 6:1982 coho smoltification study: plasma unsaturated free fatty acid levels. (A) Myristoleic acid (open circles), palmitoleic acid (open squares) and palmiteleic acid (open diamonds). (B) Oleic acid (open circles), linoleic acid (open squares) and linolenic acid (open diamonds). (C) Eicosadienoic acid (open circles) and eicosatrienoic acid (open squares) on the left axis, and docosahexaenoic acid (open diamonds) on the right axis. (D) Total unsaturated free fatty acids. Carbon numbers and number of double bonds are shown for each acid on the appropriate curve. Each point represents the mean (n=8-10) ± S.E.M. Plasma values for A, B, and C are in ng FFA/μl plasma whereas D is expressed as μmoles FFA/ml plasma. See text for detailed discussion.


A.
ng/ul plasma
C16:1t
C16:1c
C14:1
TIME (weeks)


B.
ng/ul plasma
C18:1
C18:3
C18:2
TIME (weeks)

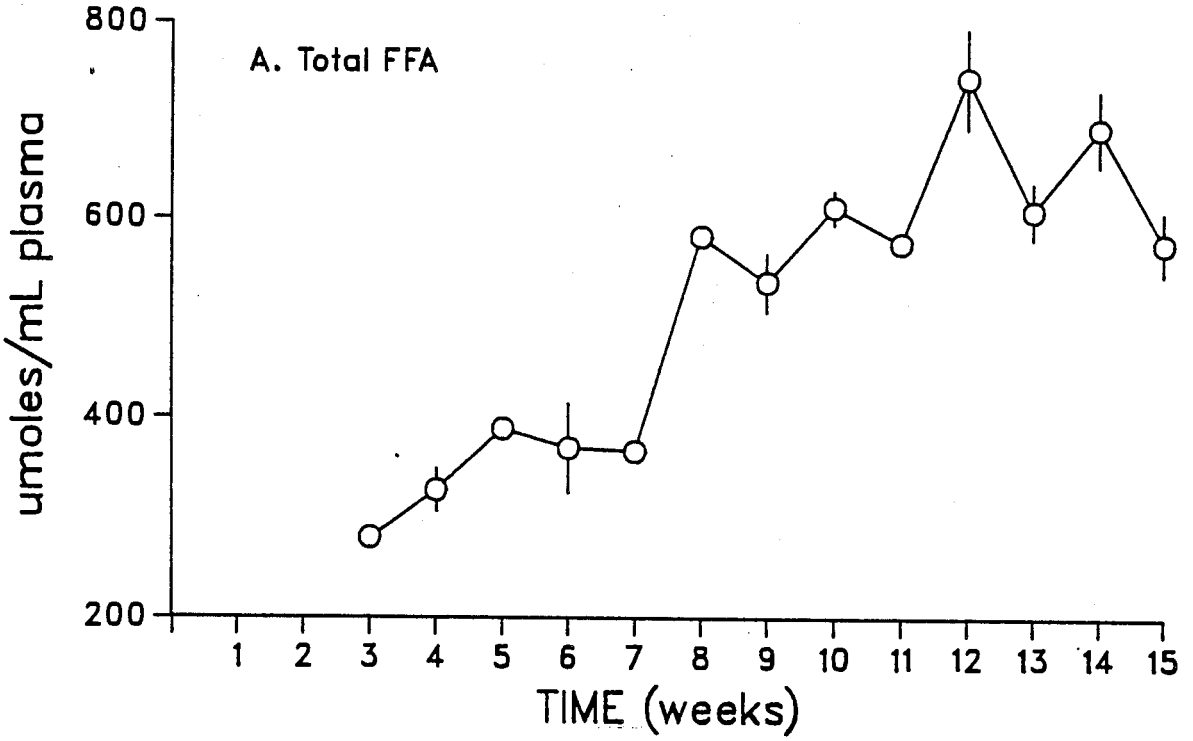

C.
ng/ul plasma
ng/ul plasma
C22:6
C20:2
C20:3
TIME (weeks)

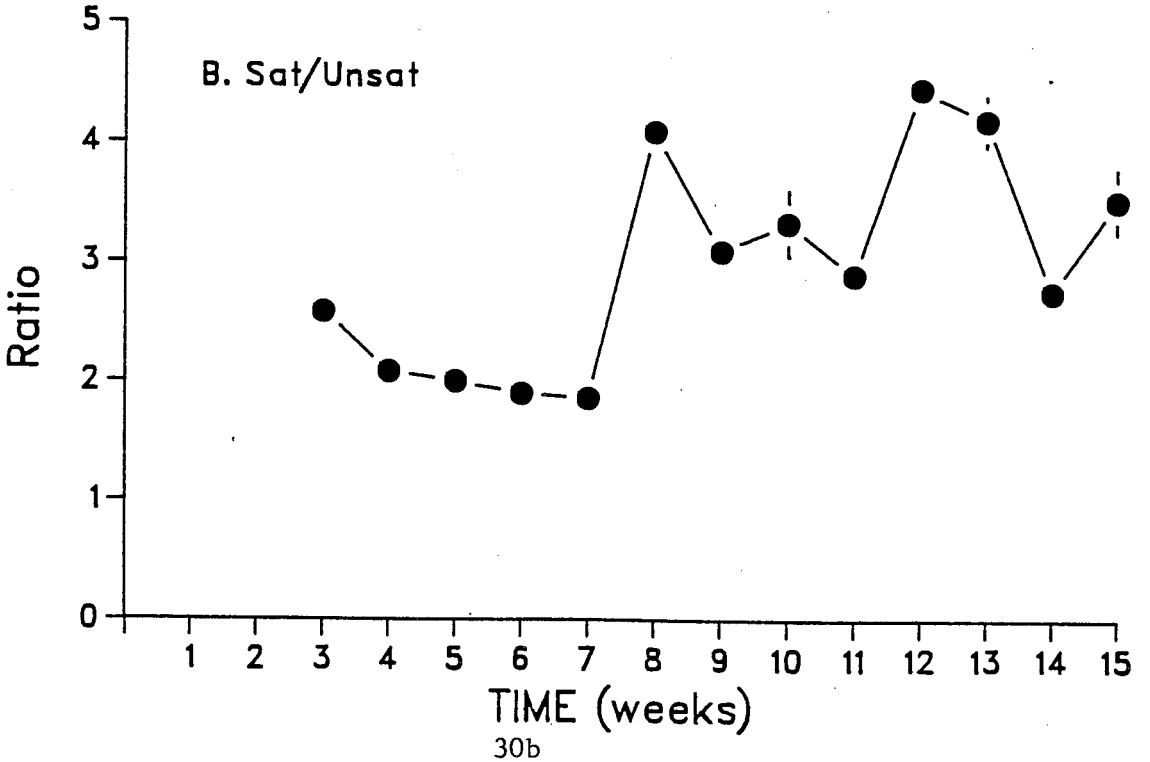

D
umoles/mL plasma
TIME (weeks)

greater than seen in all other weeks, except those from weeks 8 and 11. The week 14 value of eicosatrienoic acid (C20:3) was higher than the levels observed in weeks 3-6, 9, 10, 12, 13 and 15. Again, there were no other differences. Finally, plasma levels of docosahexaenoic acid (C22:6) did not exhibit any significant differences over the 15 weeks of sampling, although there was again a 'peak' value seen in the week 14 fish.

The total plasma FFA concentrations for the 1982 sampling year are shown in Figure 7a. Not surprisingly, there was a large increase in concentration observed between weeks 6 and 7, due primarily to the large increase in satFFA at this time. Even in weeks 3-7 (ie.,prior to the increase), the satFFA component was 2-fold higher than the unsatFFA concentration (see Figs. 5d and 6d). The mean plasma FFA levels seen in weeks 10 to 15 were higher than the levels over the initial 4 weeks. Fish sampled in week 12 exhibited the highest plasma total FFA concentration, being higher than the totals seen in weeks 3-7, 9, 10, 13 and 15. The last plot for the 1982 sampling experiment (Fig 7b) is the ratio of saturated FFA to unsaturated FFA in the plasma. Relatively stable over the first 5 weeks (weeks 3-7), the sat/unsat ratio increased dramatically in week 8 and remained elevated for most of the remaining 8 weeks of the study. The peak in plasma concentrations of several of the unsaturated fatty acids in week 14 is reflected by a large decrease in the sat/unsat ratio. A large decrease in ratio was also seen in week 11. Statistically, the ratio observed in week 12 and 13 fish was higher than the sat/unsat FFA ratios seen in the plasma of fish sampled in weeks 3-7, 9, 10, 14 and 15. The regression analysis for the plasma total FFA concentration produced a strong positive slope ($Y = 28.870X + 254.35$), as would be expected from the analyses of the saturated and unsaturated FFA. This slope was highly significant ($R^2 = .523; P < .001$). The ratio of saturated to unsaturated FFA also exhibited a very strong ($Y = 0.154X +$

Figure 7:1982 coho smoltification study. (A)Plasma total free fatty acid concentration, expressed as μmoles/ml plasma and (B)ratio of total saturated free fatty acid with total unsaturated free fatty acids. Each point represents a mean (n=8-10) ± S.E.M. See text for detailed discussion.


A. Total FFA
umoles/mL plasma
800
600
400
200
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)


B. Sat/Unsat
Ratio
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (weeks)

1.569) and significant regression (R^2 = .350;P < .001).

The interrelationships between these various morphological and plasma parameters are shown in Table 2. As expected, weight and length possessed an extremely high and positive correlation over the 15 weeks of sampling and both parameters showed strong and negative correlations with condition factor. Additionally, weight, length and condition factor all demonstrated strong correlations with plasma hematocrits. Of more interest, both weight and length demonstrated strong positive correlations with the levels of plasma GH over the duration of the study. Condition factor, an index of the relatedness of weight and length during growth, showed a significant but negative correlation with plasma GH titers. Condition factor also showed strong negative correlations to both the saturated FFA level in the plasma and the total plasma FFA. While not displaying a significant correlation with the unsaturated FFA component, condition factor did show a strong and again negative correlation to the ratio of saturated to unsaturated FFA in the plasma.

In addition to the morphological parameters, plasma hematocrit also showed a correlation with plasma glucose concentrations, and exhibited very strong and positive correlations with saturated, unsaturated and total plasma FFA, but not with the sat/unsat ratio. Additionally, saturated FFA and unsaturated FFA exhibited a strong correlation with each other.

Table 2: Correlations between parameters of the 1982 sampling period. The abbreviations are as described in the text. The correlations are one-tailed where single stars (*) represent ($P \leq .01$) and double stars (**) indicate ($P \leq .001$). See text for detailed discussion.

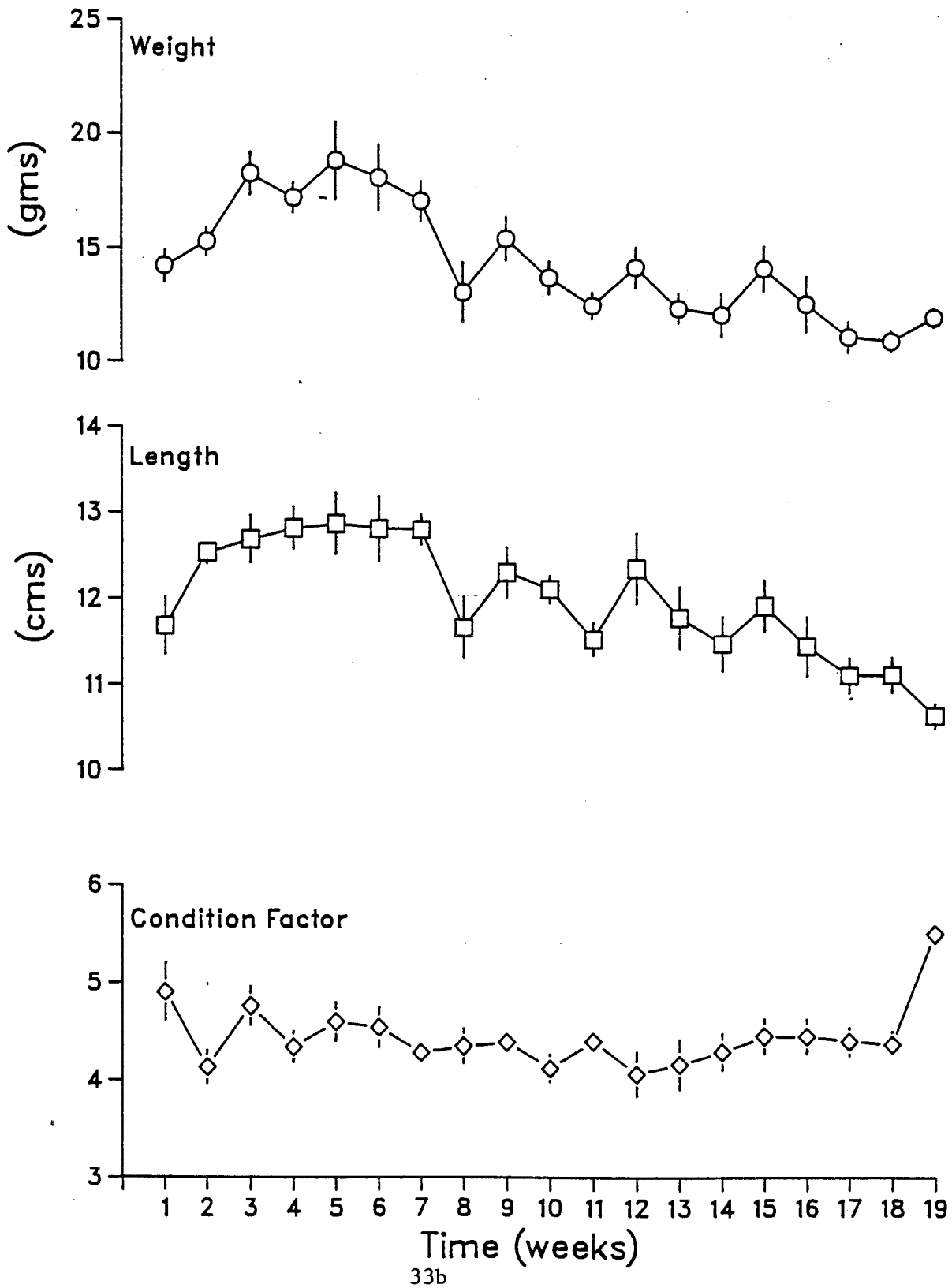
	Weight	Length	Cond. Factor	Hemato	GH	pAAN
Weight	1.0000	.9363**	-.2976**	.2784**	.3049**	-.0621
Length		1.0000	-.6023**	.4204**	.3511**	.0764
C. Factor			1.0000	-.5356**	-.2462*	.0443
Hematocrit				1.0000	.1649	-.1144
GH					1.0000	-.0999
pAAN						1.0000

	Glucose	sFFA	uFFA	Ratio S/U	tFFA
Weight	.1007	-.1106	.0267	-.1322	-.0896
Length	.1352	.1155	.0641	.0987	.1143
C. Factor	-.1311	-.5416**	-.0840	-.5637**	-.4871**
Hematocrit	.2459*	.5281**	.5110**	.1741	.5720**
GH	-.1874	.2477	.0857	.2082	.2351
pAAN	.0022	-.1979	.1137	-.2527	-.1454
Glucose	1.0000	.2934	.0938	.2126	.2748
sFFA		1.0000	.4734**	.6936**	.9811**
uFFA			1.0000	-.2809	.6351*
Ratio S/U				1.0000	.5465**
tFFA					1.0000

1983 Sampling Year

The weekly mean values for weight, length and condition factor are shown in Figure 8. Generally, fish from the initial 7 weeks of sampling (ie., prior to release from the hatchery) exhibited higher mean weights and lengths than the subsequent weeks. The mean weights from weeks 3, 5 and 6 were greater than those of week 1, 8 and 10-19 while fish sampled in weeks 4 and 7 were heavier than those sampled in weeks 8, 11-14 and 16-19. Similarly, in the length data the fish sampled in week 19 were the shortest observed and were significantly shorter than those from weeks 1-10, 12, 13 and 15. Additionally, fish from week 18 had smaller mean lengths than those from weeks 2-7, 9 and 12

Figure 8:1983 coho smoltification study: morphological data including weight (open circles), length (open squares) and condition factor (see text for explanation) (open diamonds). All points represents means (n=10) ± S.E.M. See text for detailed discussion.


Weight
(gms)
25
20
15
10
Length
(cms)
14
13
12
11
10
Condition Factor
6
5
4
3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Time (weeks)

while fish sampled in week 17 were shorter than the mean lengths observed in weeks 2-7. This disparity in weights and lengths over time was further demonstrated by regression slopes. The fish from weeks 1-7, fish tentatively termed "smolts", exhibited positive slopes for both weight (slope = 0.523) and length (slope = 0.145). However, the regression slopes seen from weeks 8 to 19, in fish tentatively undergoing desmoltification, were negative (weight, slope = -0.227;length, slope = -0.126), as were the slopes for the overall sampling period of 19 weeks (weight, slope = -0.337;length, slope = -.105). The reason for this decline in size of the fish is obviously not solely due to decreased growth rates associated with desmoltification. While decreases in weight over time are often seen in both wild and controlled environments, decreases in overall length are extremely unnatural events. It must be remembered that these values represent sub-samples of the population, and are not indicators of true growth rates as such. It seems that the method used by the hatchery to maintain a group of smolts in freshwater inadvertently led to retention of smaller individuals. These fish were externally not discernable as non-smolted fish, ie., they had full silvery coloration, absence of parr marks, and dark fin margins, all characteristic of smoltification.

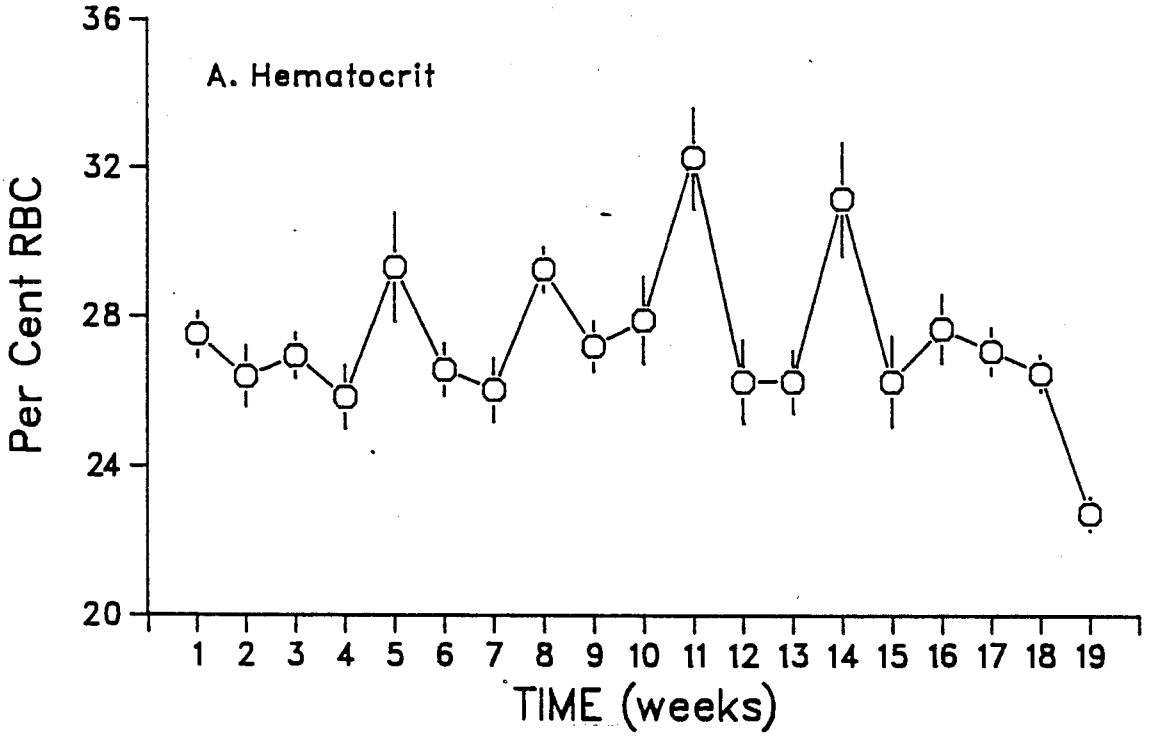
Condition factor, also shown in Figure 8, demonstrated little change over the course of the sampling, although the value of this parameter in the fish from week 19 were higher than all other weeks, perhaps an indicator that the fish were returning to parr-like forms. While there was a slight decline over the initial 7 weeks of sampling (slope = -0.044), the regression was not significant ($P > .05$). The overall slope for this parameter was positive ($Y = .0253X; P < .05$).

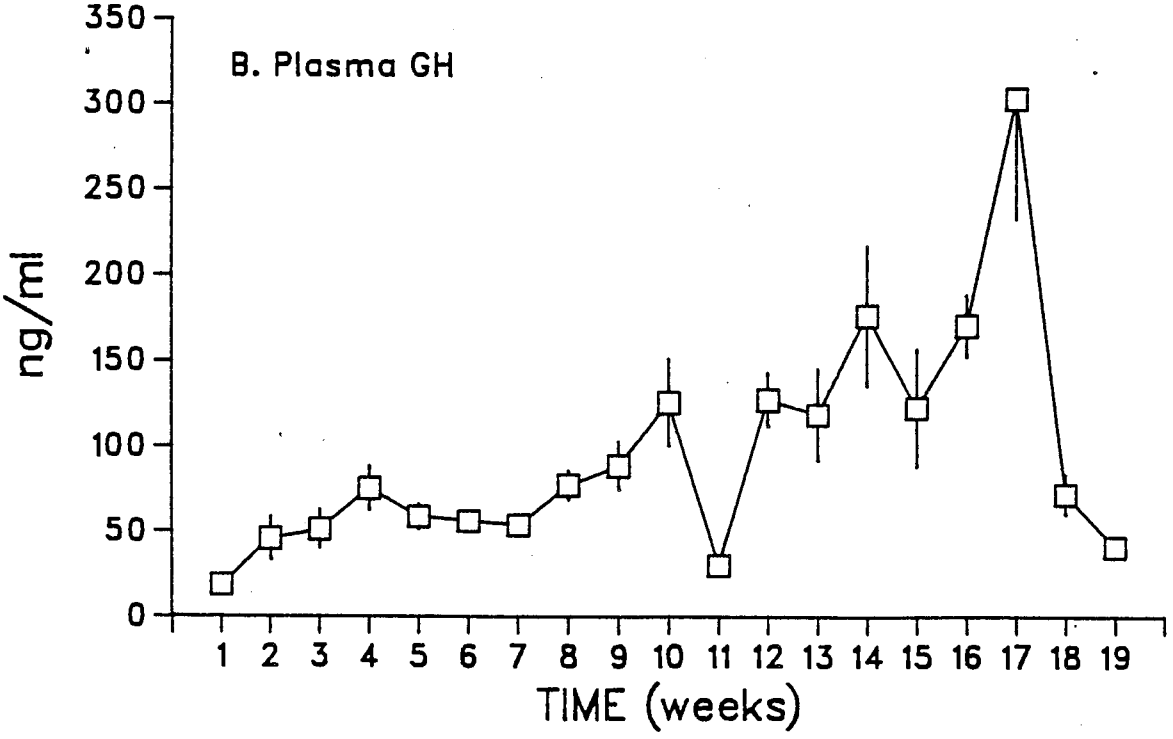
Increases in plasma hematocrits are also considered to be an indicator of smoltification. However, there were no real changes in this parameter over the sampling regime (Fig. 9a). Fish

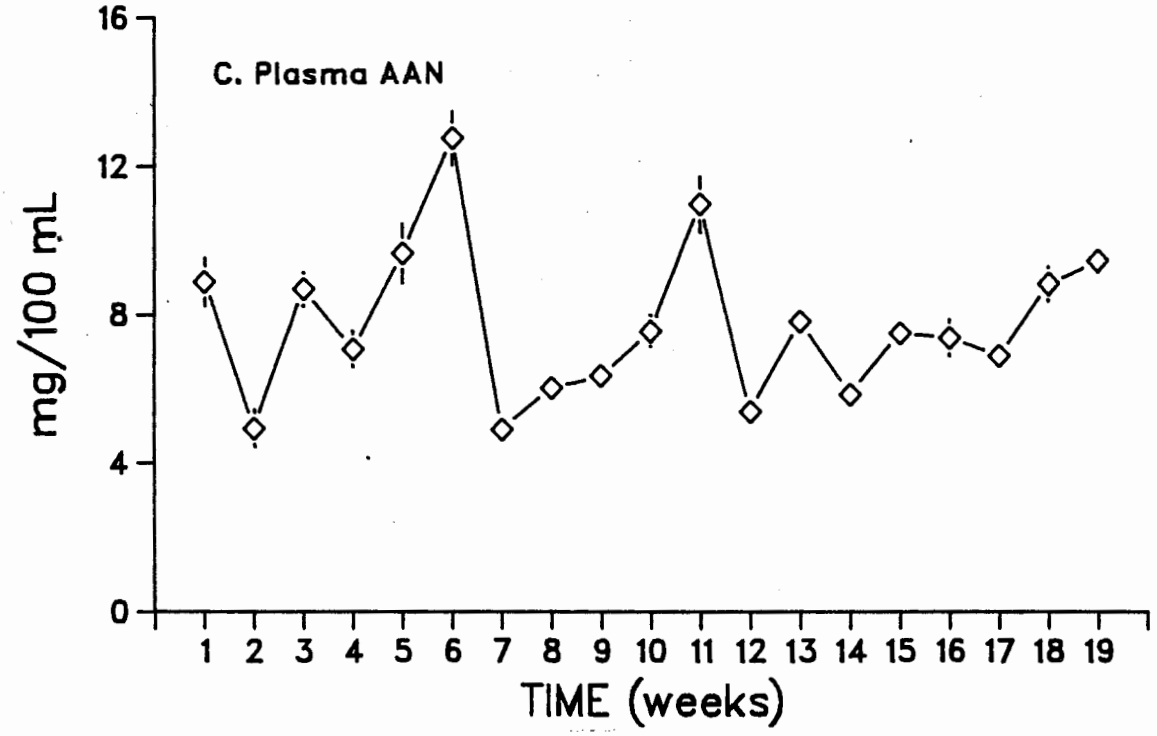
sampled in week 19 possessed lower hematocrits than all other weeks, while fish sampled in week 11 had mean hematocrits higher than those seen in weeks 1-4, 6, 7, 9, 10, 12, 13 and 15-19. Additionally, the mean hematocrit from the week 14 sample was higher than that obtained in weeks 2, 3, 4, 6, 7, 12, 13, 15, 17, 18, and 19. Plasma GH levels, shown in Fig. 9b, increased over the sampling period to a maximum at week 17 whereafter they declined to levels similar to initial values. The plasma GH level seen in week 17 was higher than all other values. Additionally, the peak seen in week 14 was higher than plasma GH titers seen in weeks 1-8, 11, 18 and 19. Week 16, just prior to the overall peak seen in week 17, had a higher plasma GH concentration than seen in weeks 1, 2, 3, 5, 6, 7, 11 and 19. Unlike the previous parameters (weight, length and condition factor), plasma GH titers increased over the entire sampling period. The regression for plasma GH levels for weeks 1-7 ($Y = 4.74X + 31.87$; $R^2=.0896$;$P = .006$) and weeks 1-19 ($Y = 5.622X + 34.99$; $R^2=.1238$;$P < .001$) were quite similar, whereas the post-release fish (weeks 8-19) had very different values ($Y = 2.762X + 77.55$; $R^2=.0093$;$P > .05$).

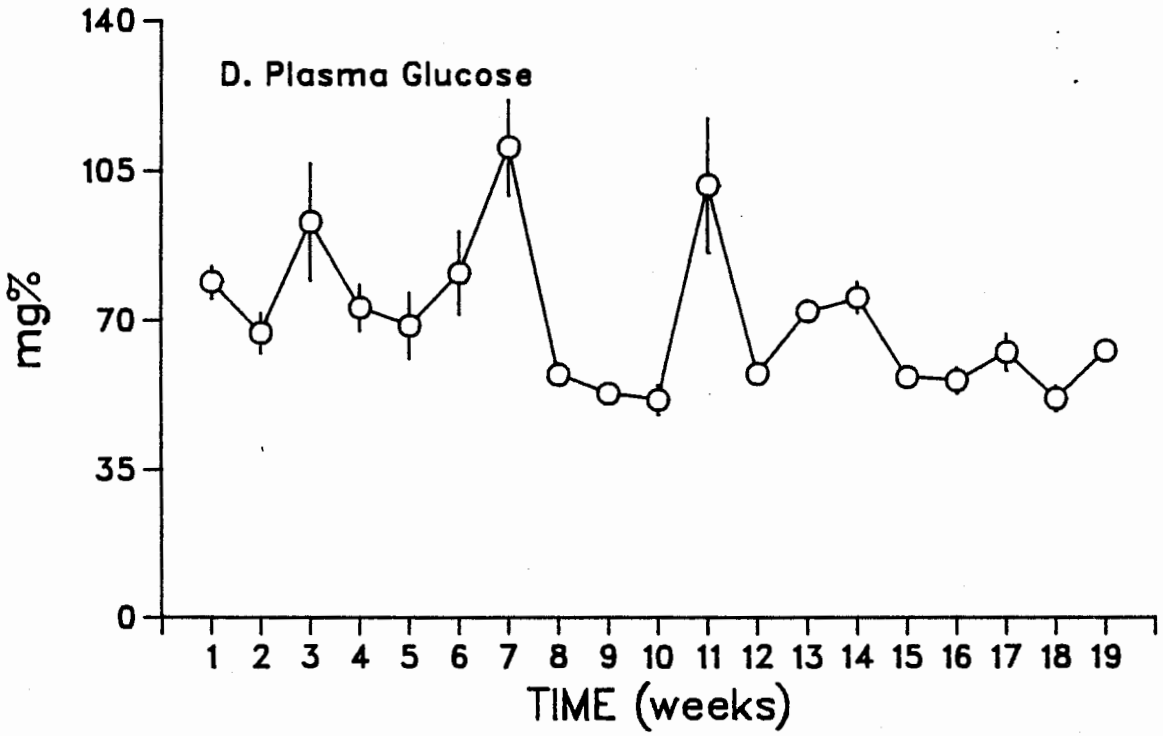
Plasma glucose levels for this experiment are shown in Fig. 9d. This parameter demonstrated relatively wide fluctuations over the sampling period, with large peaks seen at weeks 3, 7, and 11. These peaks occurred immediately following full moons of the lunar cycle. The largest peak, seen in week 7, was higher than all other values, with the exception of the plasma glucose titers observed in weeks 3 and 11. The concentration of glucose in the plasma from the week 11 fish was higher than those levels seen in weeks 2, 4, 5, 8, 9, 10, 12 and 15-19, while the third major peak, that of week 3, was higher than the concentrations seen in weeks 8-10, 12 and 15-19. The plasma amino acid nitrogen (pAAN) concentrations also demonstrated dramatic fluctuations over the 19 sampling weeks (Fig. 9c). Like plasma glucose, the peak 'fluctuations' (weeks 3, 7 and 11) occurred close to full moons

Figure 9:1983 coho smoltification study: plasma hematocrits (A), GH levels (B), amino acid nitrogen levels (C), and glucose levels (D). Each point represents the mean (n=8-10) ± S.E.M. Hematocrits are expressed as percent, GH values are expressed as ng GH/ml plasma, and both amino acid nitrogen and glucose are expressed as mg/100 ml plasma. See text for detailed discussion.


A. Hematocrit
Per Cent RBC
36
32
28
24
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


B. Plasma GH
ng/ml
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


C. Plasma AAN
mg/100 mL
16
12
8
4
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


D. Plasma Glucose
mg%
140
105
70
35
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)

(although these were large decreases rather than the large increases noted for plasma glucose). The highest pAAN level observed, in week 6, was greater than all other observed concentrations with the exceptions of weeks 5 and 11. Fish sampled in weeks 5 and 19 possessed higher pAAN levels than those from weeks 2, 4, 7-10, 12 and 14-17. Further, the mean concentrations of pAAN observed in fish from weeks 1, 3 and 18 were all higher than those observed in weeks 2, 7, 8, 9, 12 and 14. Regression analysis of the plasma AAN data was nonsignificant whereas plasma glucose data showed a strong ($P < .001$) negative regression ($R^2 = .0835$; $Y = -1.224X + 82.48$).

Mean plasma levels of free fatty acids for the 1983 sampling year are presented in Figures 10-12. There were no plasma free fatty acids analysed for the week 19 fish. The total saturated free fatty acid concentration for the 18 weeks is shown in Fig. 10d. The large peak value observed in the week 16 fish was higher than all other weekly values. The levels of satFFA seen in weeks 15 and 17 (ie., on either side of the peak) were also higher than levels measured in weeks 1,3-12 and 18. In the initial 7 weeks sampling (representing the final stages of smoltification) the regression for the saturated FFAs was positive ($Y = 3.785X + 81.334$) but not significant ($P = 0.06$). The slope of this parameter was greatly enhanced in the post-release weeks ($Y = 13.826X - 30.437$) and was highly significant ($R^2 = .2001$; $P < .001$). Over the entire 18 weeks, the regression for saturated FFAs was also significant ($Y = 7.423X + 59.172$; $R^2 = .2407$; $P < .001$). The individual satFFAs

The large peak in total satFFA in weeks 15 and 16 were also observed in the individual satFFAs, shown in Figs. 10a-c.[2] There was a small peak in plasma concentration of lauric acid (C15:0), centred around week 15, which was higher than all other weekly means except that seen in weeks 14 and 16. Week 13 fish also

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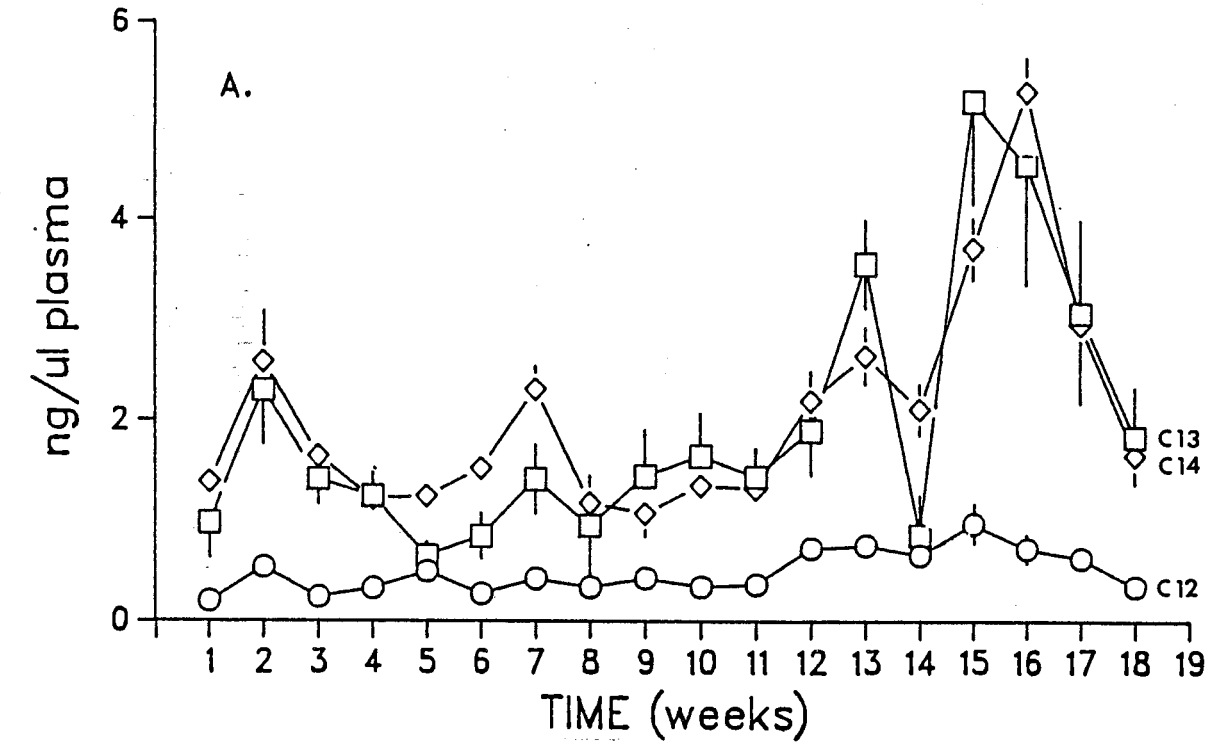
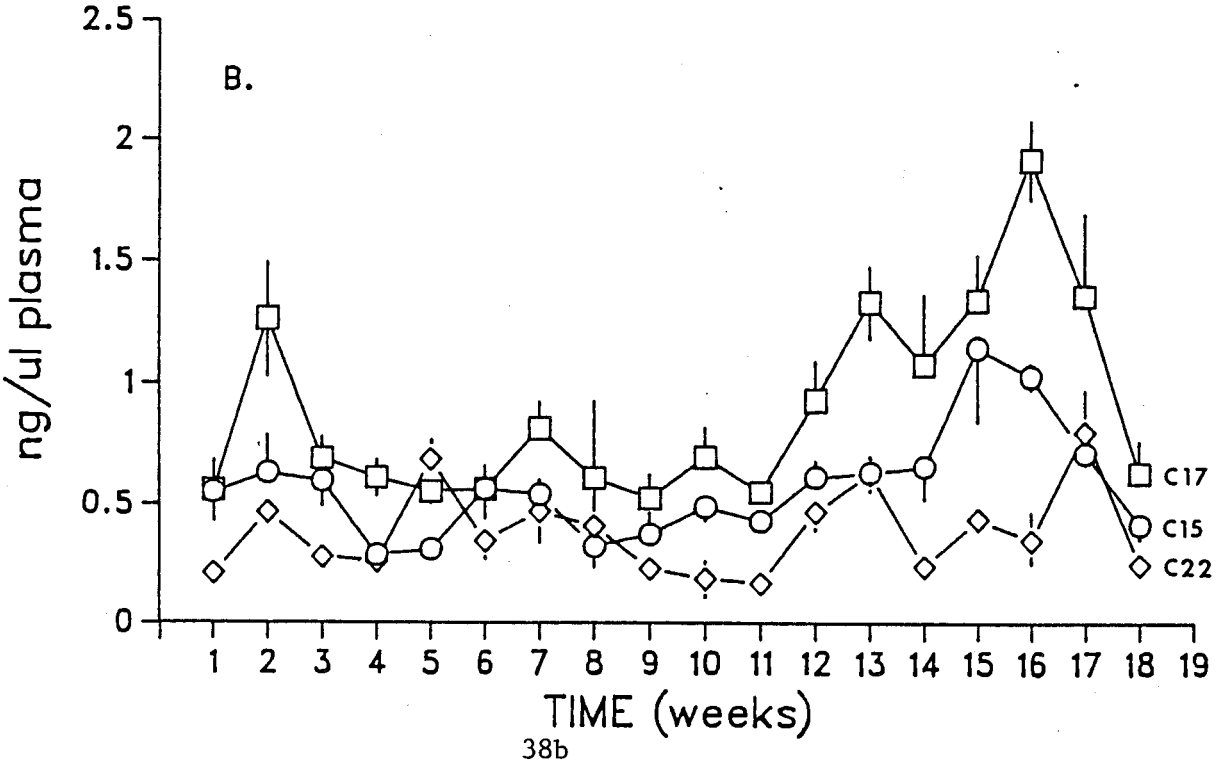
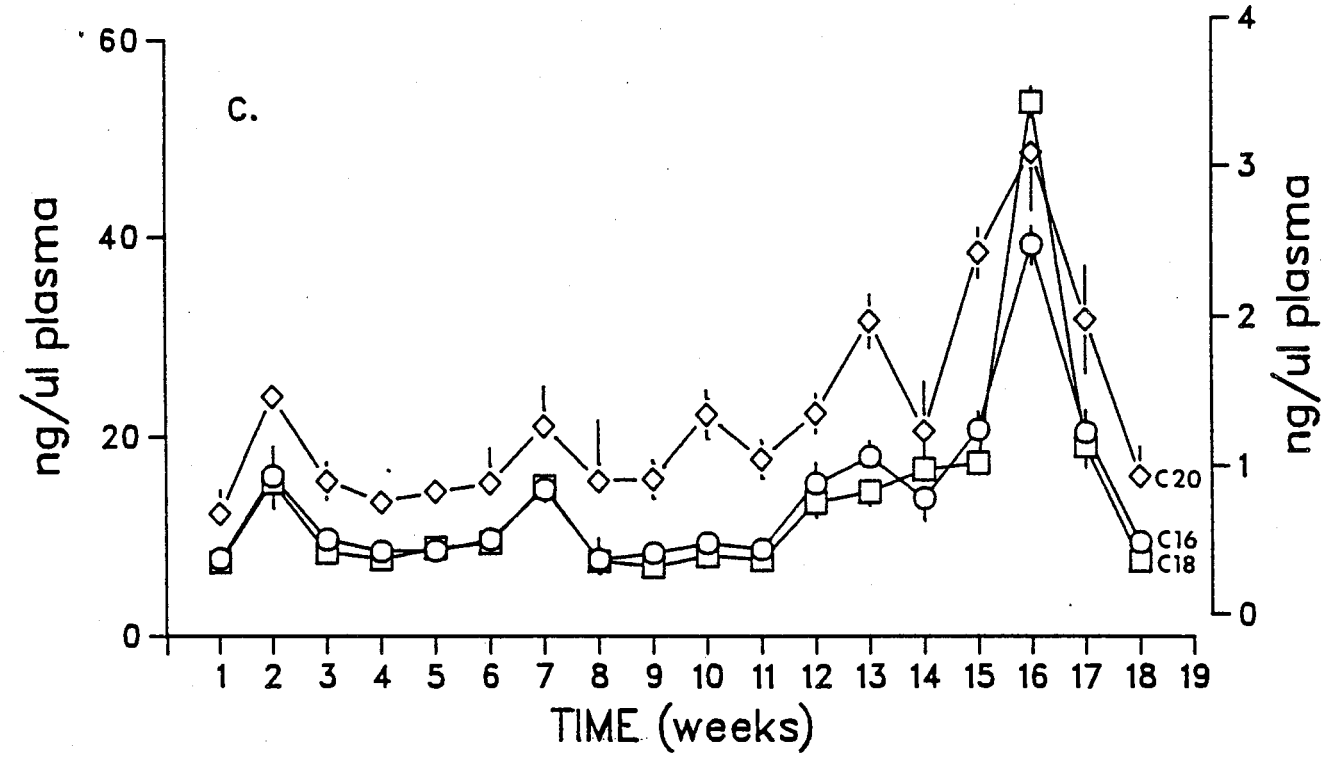
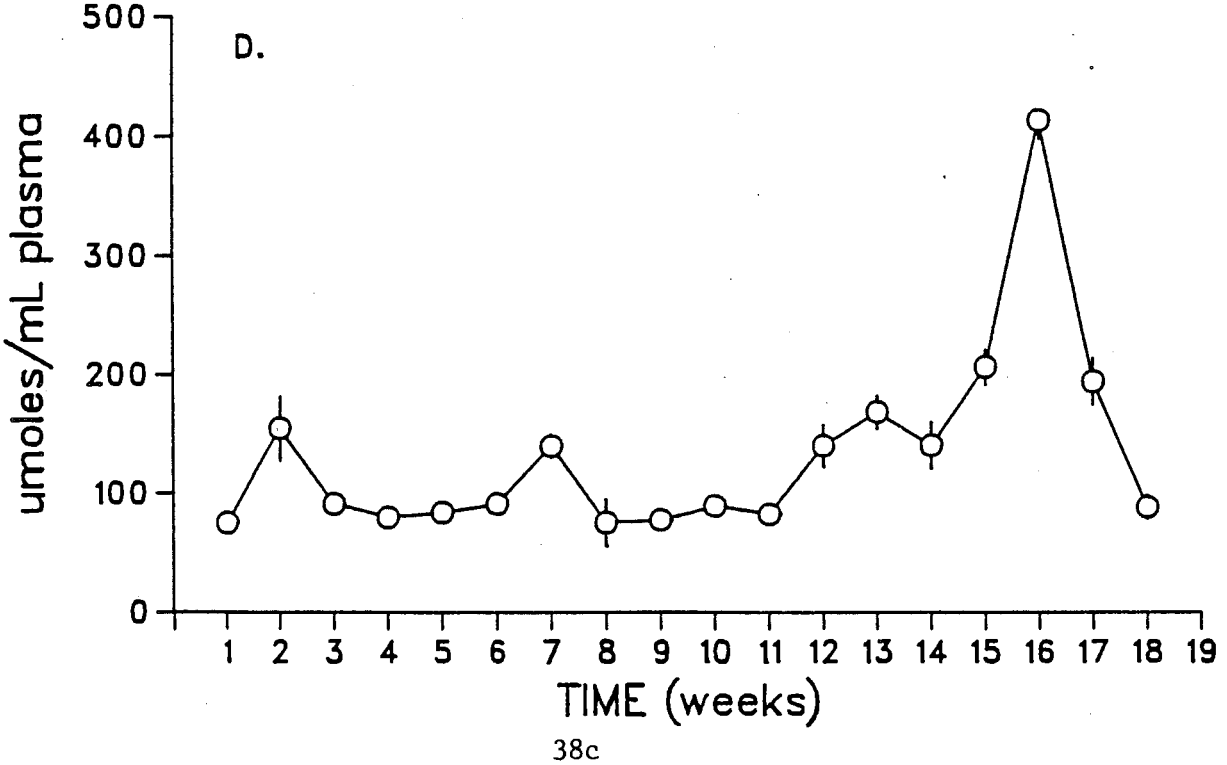
[2]See Table 1 for detailed description of fatty acid nomenclature.

Figure 10:1983 coho smoltification study: plasma saturated free fatty acid levels. (A) Lauric acid (open circles), tridecanoic acid (open squares) and myristic acid (open diamonds). (B) Pentadecanoic acid (open circles), heptadecanoic acid (open squares) and arachidic acid (open diamonds). (C) Palmitic acid (open circles), stearic acid (open squares) and behenic acid (open diamonds). (D) Total saturated free fatty acids. Carbon numbers are shown for each acid on the appropriate curve. Each point represents the mean (n=8-10) ± S.E.M. Plasma values for A, B, and C are in ng FFA/μl plasma whereas D is expressed as μmoles FFA/ml plasma. See text for detailed discussion.


A.
ng/ul plasma
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)
C13
C14
C12


B.
ng/ul plasma
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)
C17
C15
C22


C.
ng/ul plasma
ng/ul plasma
C20
C16
C18
TIME (weeks)


D.
umoles/mL plasma
TIME (weeks)

exhibited a small peak which was higher than the levels seen in fish sampled in weeks 1, 3, 4, 6-11 and 18. Further, week 12 fish had higher plasma lauric acid levels than fish from weeks 1, 3, 4, 6, 10, 11 and 18. Plasma levels of tridecanoic acid (C13:0) in week 15 fish were also higher than any other weekly mean, except that of week 16 fish. In turn, the week 16 fish possessed higher plasma C13:0 levels than fish sampled in weeks 1, 3-12, 14 and 18. The peak values of myristic acid (C14:0) observed in weeks 15 and 16 were higher than all other values. Additionally, fish sampled in weeks 13 and 17 possessed higher plasma myristic acid concentrations than did fish sampled in weeks 1, 3-6, 8-11 and 18.

Plasma concentrations of pentadecanoic acid (C15:0)(Fig. 10b) again showed a peak value in week 15 fish. This concentration was higher than seen in any other week, except weeks 2 or 16. The week 16 value of pentadecanoic acid was also higher than seen in fish from weeks 4, 5, 8, 9, 11 or 18. The peak in heptadecanoic acid (C17:0) was centred on week 16. As with C15:0, this value was higher than seen in all other weeks with the exception of weeks 2 and 13. The concentrations from these two weeks were higher than observed in fish from weeks 1, 3-6, 9, 11 and 18. The third curve plotted in Figure 10b represents behenic acid (C22:0). This fatty acid displayed two large peaks in concentration. The week 17 concentration was higher than most weeks, but not higher than levels seen in weeks 2, 5 or 8 whereas the fish sampled in week 5 possessed higher plasma behenic acid levels than fish sampled in weeks 1, 3, 4, 6, 9-11, 14 and 18.

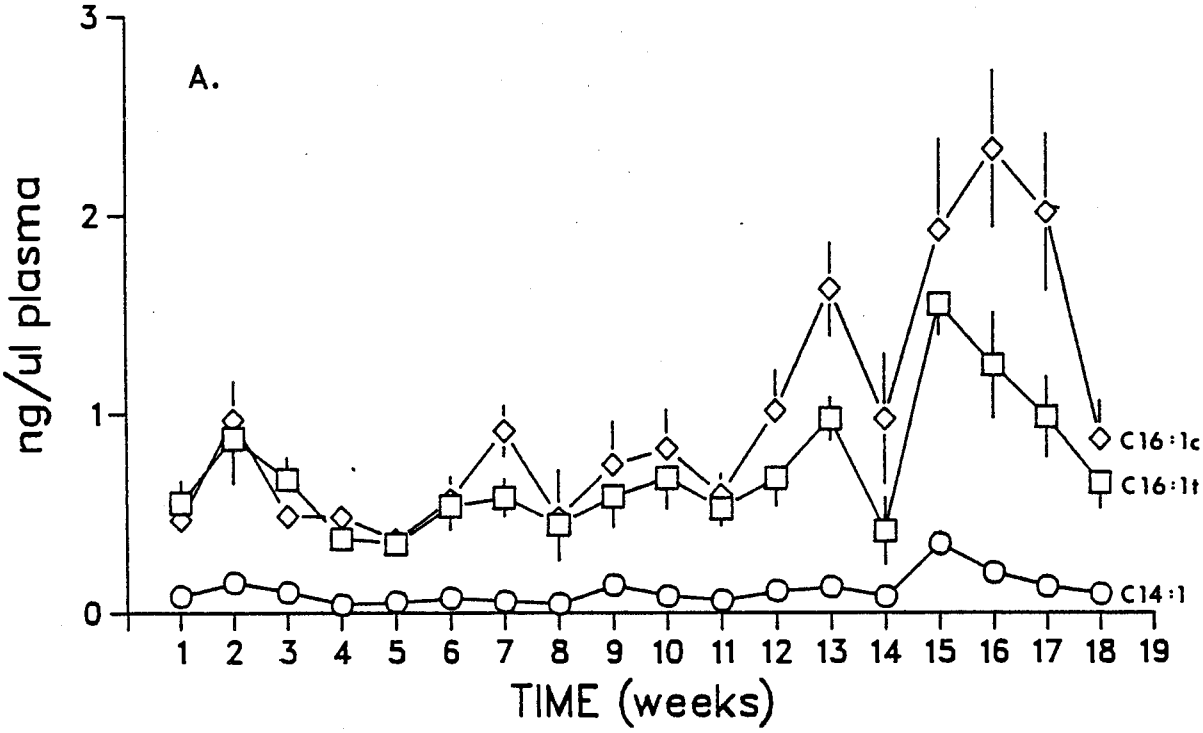
The last 3 saturated fatty acids assayed are plotted in Figure 10c. As seen in the 1982 data, palmitic (C16:0) and stearic (C18:0) acids represent a large proportion of the total FFA concentration. Both palmitic and stearic acid concentrations peaked dramatically in the fish sampled in week 16. In both cases, the week 16 value was very significantly higher than all

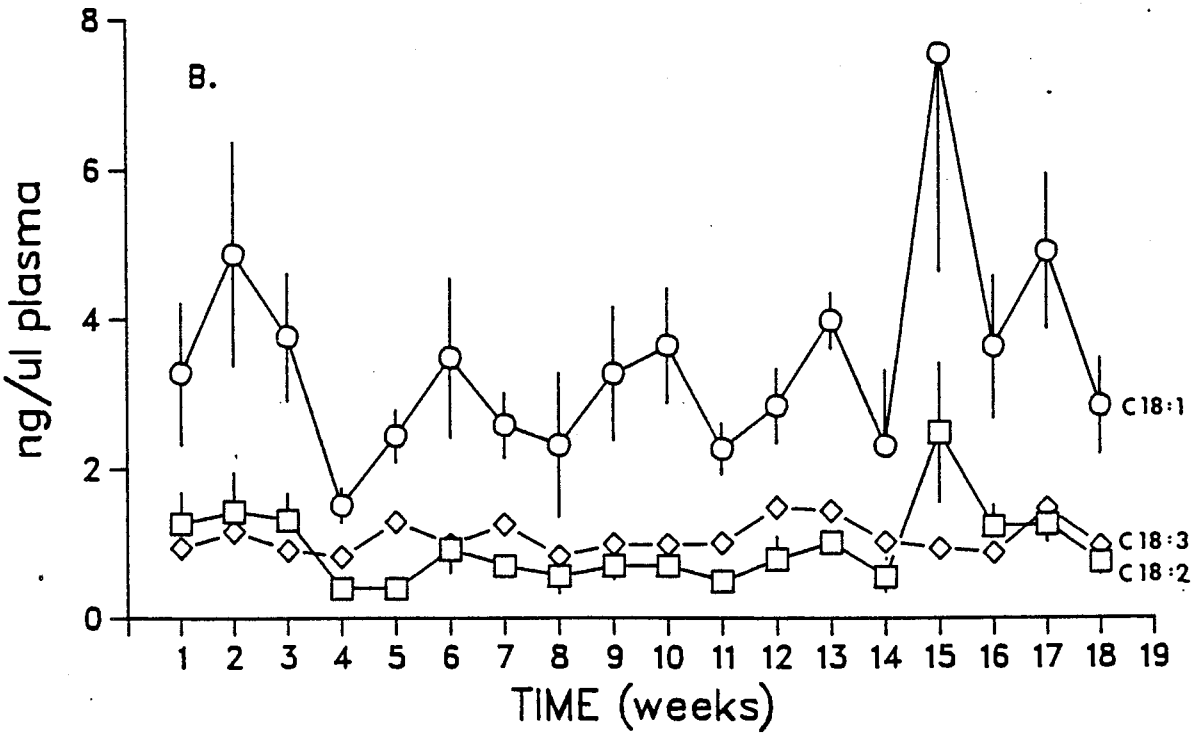
other weekly levels. Additionally, the C16:0 levels seen in fish from weeks 13, 15 and 17 were higher than measured in weeks 1, 3-6, 8-11, and 18. Plasma levels of stearic acid in fish sampled in week 17 were higher than levels observed in weeks 1, 3-6, 8-13 and 18. Further, the mean plasma concentrations of C18:0 in fish sampled in weeks 2, 7 or 12-15 were all higher than the levels seen in weeks 1, 3-6, 8-11 and 18. The week 16 peak in arachidic acid (C20:0) was again higher than all other values for this parameter. Further, the plasma level of arachidic acid in week 15 fish was higher than that of fish sampled in weeks 1-12,14 and 18. Lastly, fish sampled in week 13 displayed higher plasma concentrations of this fatty acid than did fish from weeks 1, 3-6, 8, 9, 11, 12 and 18.

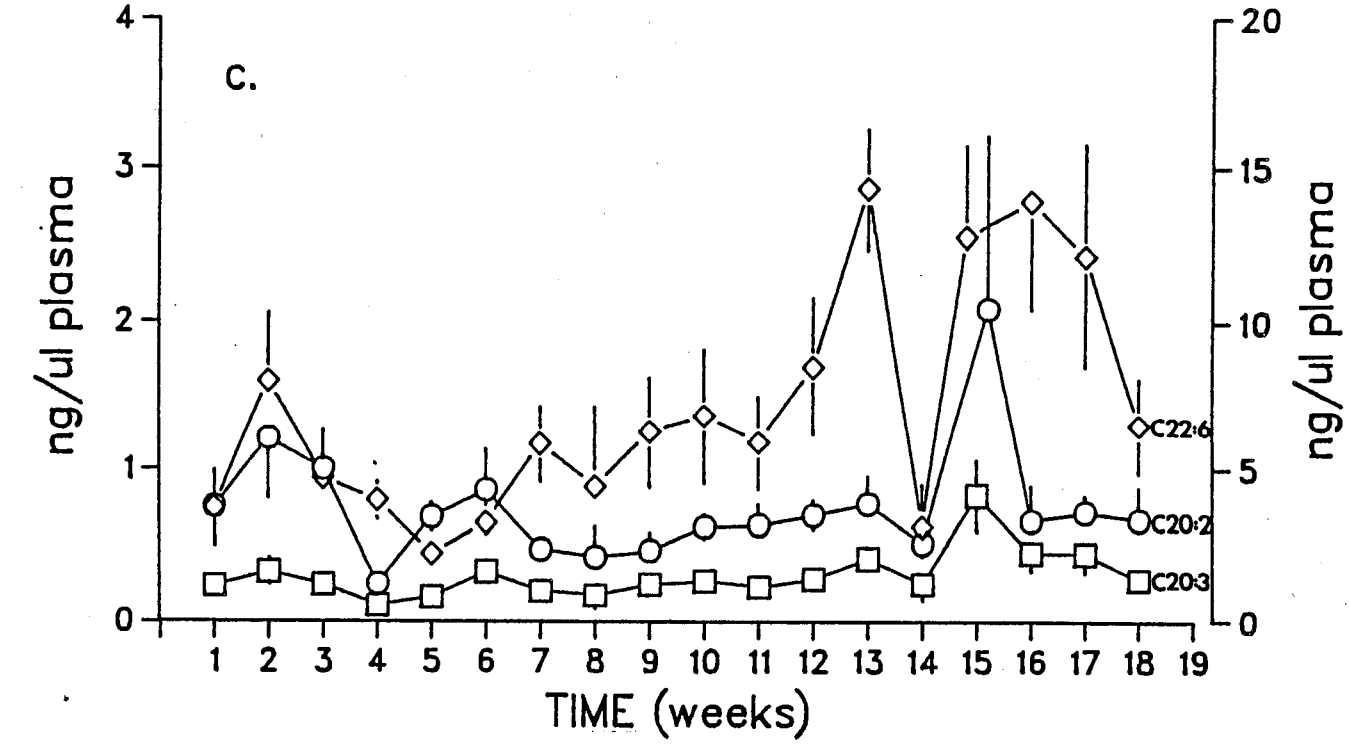
The unsaturated FFA profiles for these weekly samples are shown in Figure 11a-d. As in their saturated counterparts, many of the unsaturated FFAs increased in the post-release fish and the week 15 value in total unsatFFA (Fig. 11d) was higher than the levels measured in weeks 1, 3-12, 14 and 18. The regression slope in the 'smolt' portion of the sampling regime (ie., weeks 1-7) was nonsignificant, but over the desmolt period (weeks 8-18) the regression was significant (R^2 = .076;P = .010) and positive (Y = 3.44X + 15.324). The regression analysis for the entire 18 weeks was also positive (Y = 2.45X + 28.152) and highly significant (R^2 = .1448;P < .001).

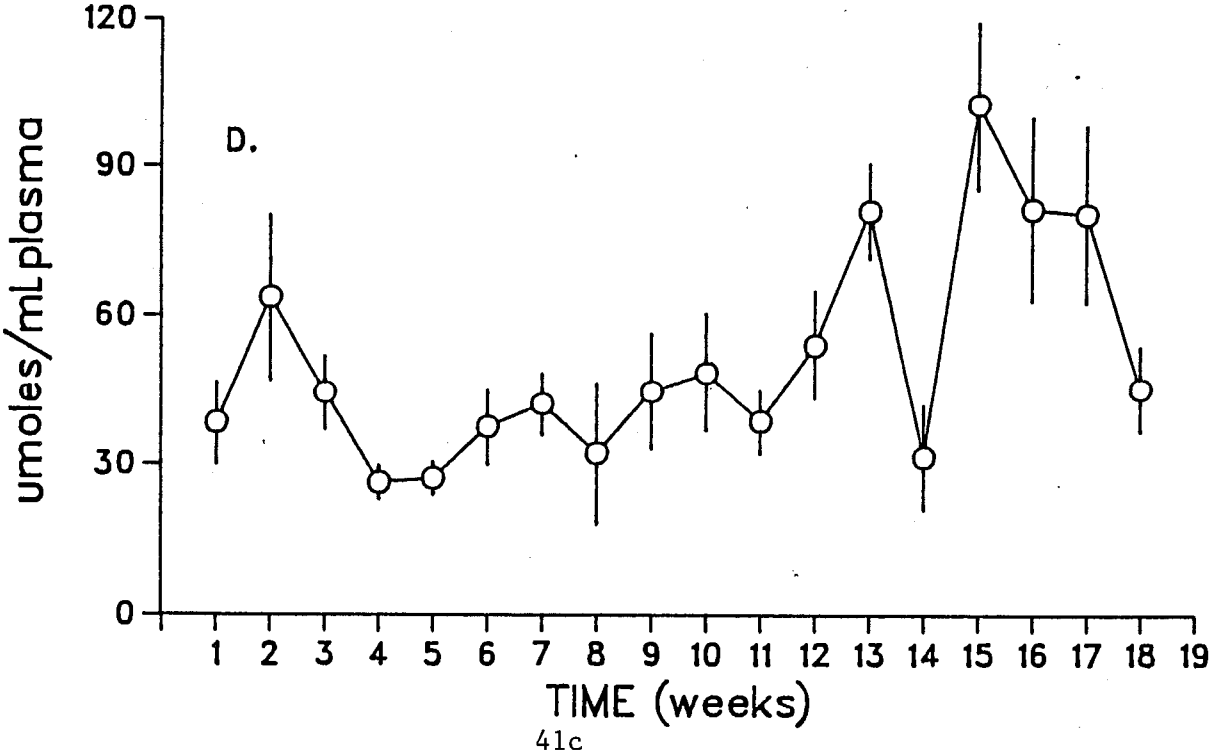
As with the satFFAs, the pattern of total unsatFFA was seen in most of the individual unsatFFAs (Figs. 11a-c). Myristoleic (C14:1)and palmitoleic (C16:1t) acids both display a peak value in week 15 which was higher than all other weeks. The week 16 levels for both acids were also higher than weeks 4-8 and 11, with the C16:1t value also higher than weeks 1 and 14. Palmiteleic acid, the *cis* form of C16:1, had high plasma concentrations spanning weeks 15 to 17 which were higher than seen in weeks 1, 3-12 and 14. As well, the level of C16:1c

Figure 11:1983 coho smoltification study: plasma unsaturated free fatty acid levels. (A) Myristoleic acid (open circles), palmitoleic acid (open squares) and palmiteleic acid (open diamonds). (B) Oleic acid (open circles), linoleic acid (open squares) and linolenic acid (open diamonds). (C) Eicosadienoic acid (open circles) and eicosatrienoic acid (open squares) on the left axis, and docosahexaenoic acid (open diamonds) on the right axis. (D) Total unsaturated free fatty acids. Carbon numbers and number of double bonds are shown for each acid on the appropriate curve. Each point represents the mean (n=8-10) ± S.E.M. Plasma values for A, B, and C are in ng FFA/µl plasma whereas D is expressed as µmoles FFA/ml plasma. See text for detailed discussion.


A.
ng/ul plasma
3
2
1
0
C16:1c
C16:1t
C14:1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


B.
ng/ul plasma
8
6
4
2
0
C18:1
C18:3
C18:2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


C.
ng/ul plasma
ng/ul plasma
C22:6
C20:2
C20:3
TIME (weeks)


D.
umoles/mL plasma
TIME (weeks)

observed in week 13 was higher than seen in weeks 1, 3-6, 8, 11, and 12.

The 18 carbon unsaturated FFA plasma concentrations are shown in Fig. 11b. Oleic acid, the monounsaturated form, displayed a higher value in week 15 fish than was seen in fish from weeks 3, 4, 5, 7, 11, 12, 13 and 18. Linoleic acid, or C18:2, also displayed only one major elevation in plasma concentration, again seen in week 15 fish. This value was greater than observed in all other weeks, except weeks 13 and 16. Unlike the rest of the unsatFFA discussed thus far, linolenic acid (C18:3) did not show an elevation in plasma concentration in week 15. Indeed, the week 15 value was one of the lowest observed. Plasma levels of this FFA in week 12 fish were higher than levels seen in fish from weeks 1, 3, 4, 6, 8-11, 14, 15, 16 and 18. Plasma levels of linolenic acid in fish sampled in week 13 were higher than the levels seen in weeks 1, 3, 4, 6, 8, 9, 11, 14, 15, 16 and 18 and the value measured in week 17 fish was higher than observed in weeks 1, 3, 4, 6, 8, 9, 11, 15, 16 and 18.

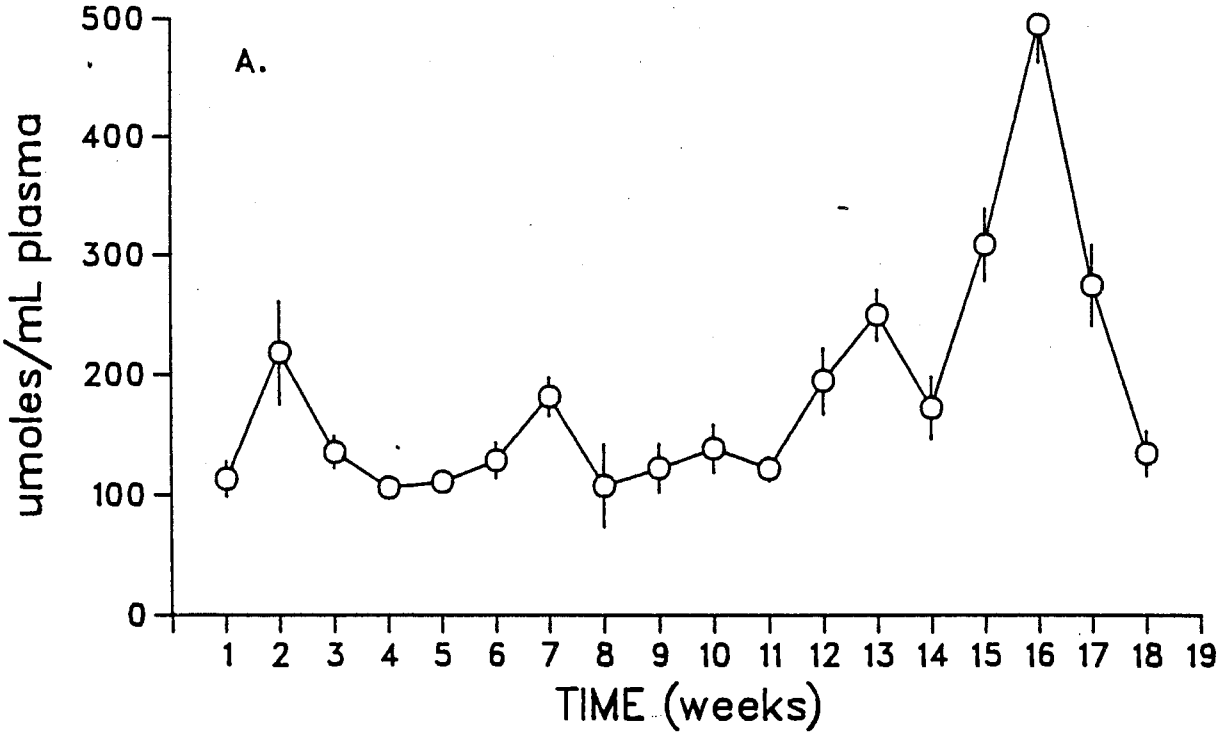
Plasma profiles of the final 3 unsaturated FFAs examined are shown in Fig. 11c. Although the value for eicosadienoic acid (C20:2) in fish from week 15 was quite high, the large variation limits its significance. Thus, the value is higher only than levels seen in weeks 3, 4, 6, 12 and 13. However, the week 15 value of eicosatrienoic acid (C20:3) was higher than recorded in all other weeks. The final unsaturated FFA examined was C22:6, or docosahexaenoic acid (Fig. 11c). The plasma concentration of this FFA was among the highest seen for the various unsaturated FFAs. The level of this FFA in week 13 fish was higher than concentrations seen in fish from weeks 1, 3-7, 11, 14 and 18. As well, week 16 fish possessed plasma levels of C22:6 which was greater than seen in fish sampled in weeks 1, 3, 4, 5 and 6.

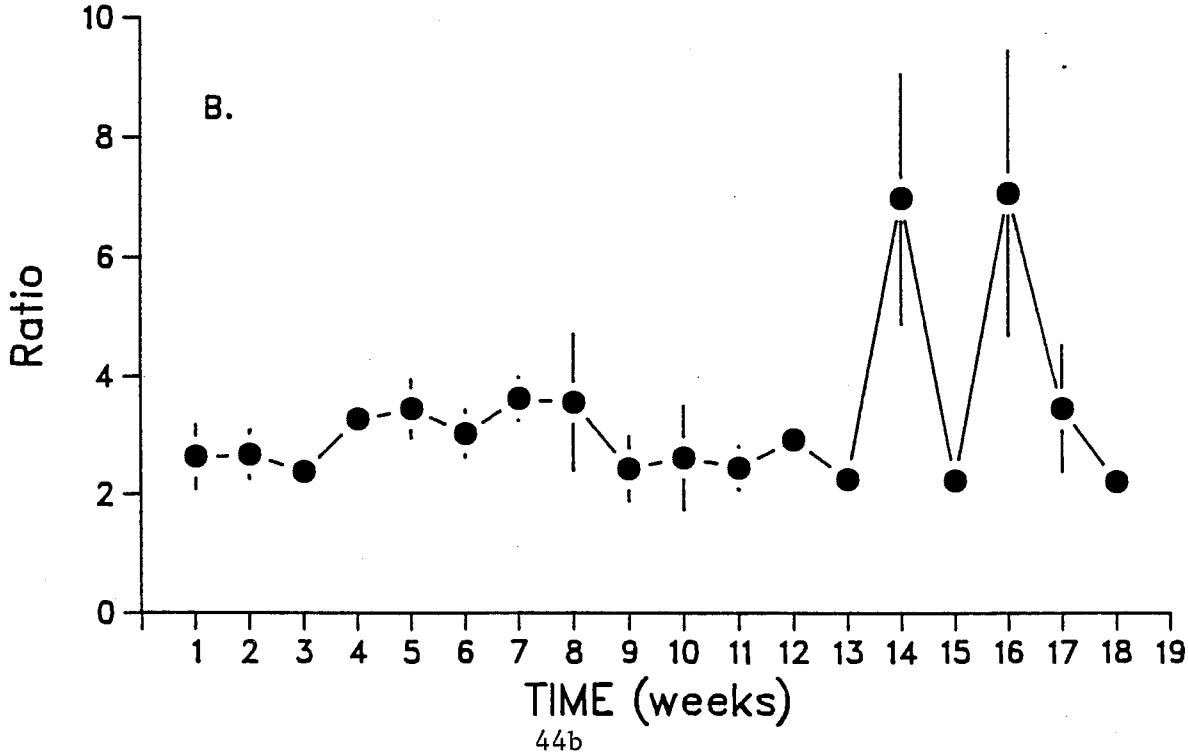
The total FFA content in the plasma of these fish in shown in Figure 12a. As would be expected from the previous results, the concentrations were considerably higher in the post-release fish. Week 16 fish had higher total FFA levels than any other week. Additionally, fish sampled in weeks 15 or 17 had greater total FFA titers than did fish sampled in weeks 1, 3-12, 14 or 18. Lastly, week 13 fish possessed plasma levels of FFA which were higher than the levels of FFA seen in fish from weeks 1, 3-6, 8-11 or 18. As in both satFFA and unsatFFA, the regression in the first 7 weeks was not significant. However, the slope increased dramatically in the post-release weeks ($Y = 17.266X - 15.113$) and was highly significant ($R^2 = .1899; P < .001$). Overall, the slope for this parameter was positive ($Y = 9.874X + 87.324$) and significant ($R^2 = .2452; P < .001$).

The ratio of saturated FFA to unsaturated FFA in the plasma is shown in Figure 12b. There were two large peaks observed for this parameter, one in fish from week 14 and one in fish sampled in week 16. Both peak values were significantly higher than observed in any other week. The large decrease in ratio seen in the week 15 fish is due primarily to increased unsatFFA plasma concentrations seen in these fish. The week 16 value, in turn, is greatly influenced by one member of this sample which possessed extremely low circulating levels of unsaturated FFAs while the saturated FFA titers were quite normal. The ratio of saturated to unsaturated FFA exhibited different regression patterns than either of its components, or of the total FFA concentration. In the initial 7 weeks of sampling, the regression for ratio was significant ($R^2 = .0816; P = .020$) with a positive slope ($Y = 0.105X + 2.305$). However, neither the post-release regression or the overall regression were significant ($P > .05$).

Muscle glycogen levels declined over the first 10 weeks of sampling, such that values in week 10 were approximately 1/5 of the level seen in the initial week of sampling (Figure 13a). The

Figure 12:1983 coho smoltification study. (A)Plasma total free fatty acid concentration, expressed as μmoles/ml plasma and (B)ratio of total saturated free fatty acid with total unsaturated free fatty acids. Each point represents a mean (n=8-10) ± S.E.M. See text for detailed discussion.

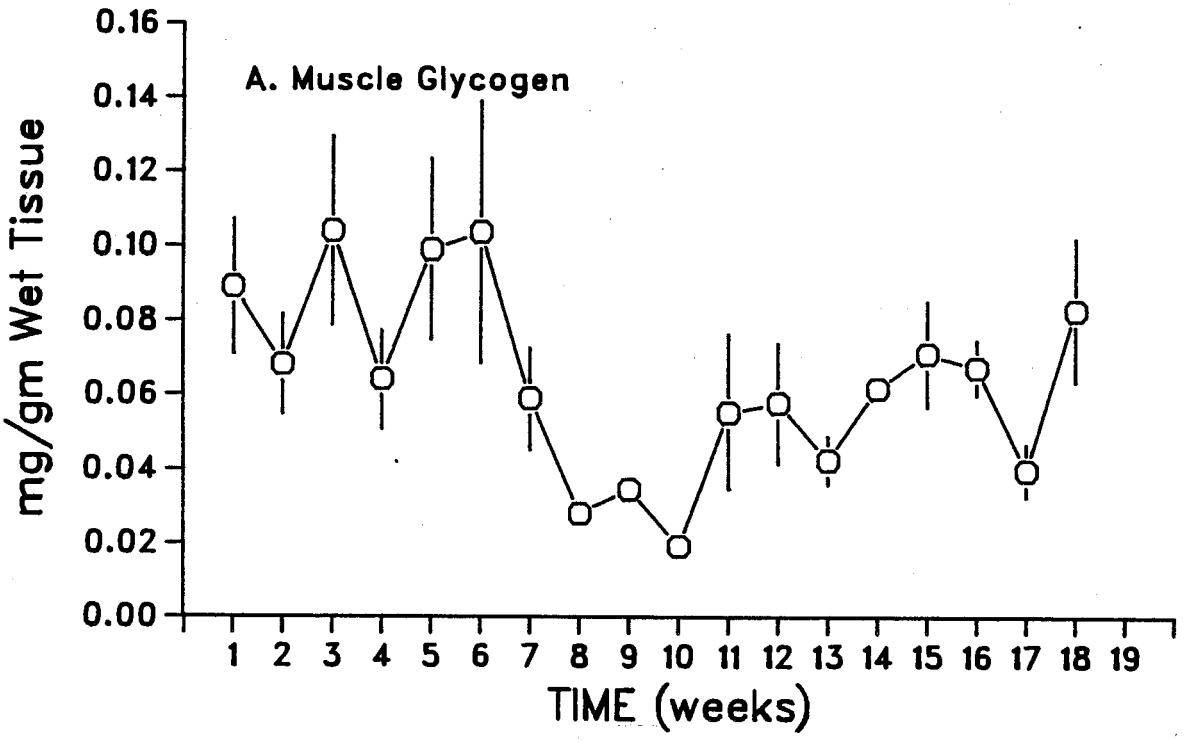

A.
umoles/mL plasma
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)

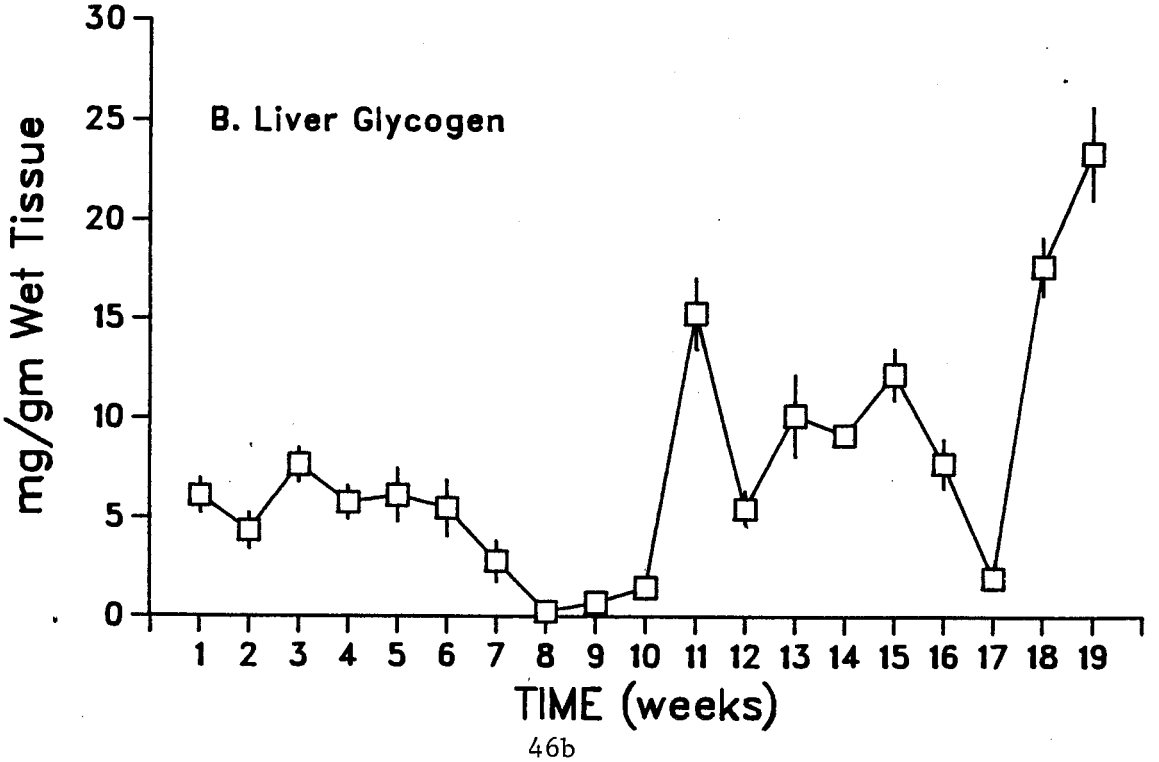

B.
Ratio
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)

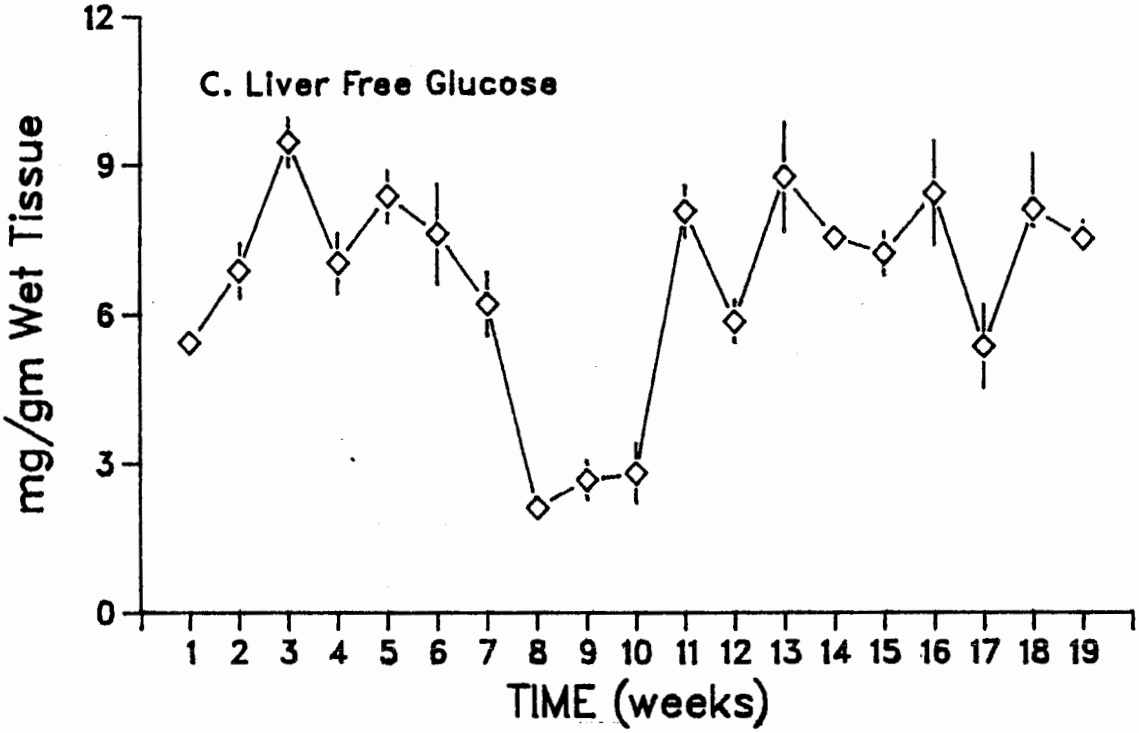
subsequent samples (ie., weeks 11 to 18) exhibited a return to the higher values associated with parr and pre-smolts. Muscle glycogen levels at week 10 were lower than values from weeks 1-7, 15, 16 and 18. The levels observed in week 8 were lower than seen in weeks 1, 3, 5 and 6. Liver glycogen values (Figure 13b) also exhibited a decline over the initial 8-10 weeks of sampling, to the extent that values from weeks 8 and 9 were almost non-detectable. As seen in the muscle compartment, desmolting fish showed a return to higher concentrations of glycogen in the hepatic tissue such that levels of liver glycogen in week 19 were higher than all other weeks. Further, concentrations of liver glycogen from weeks 18 and 11 were higher than values observed from weeks 1-17. Conversely, the values seen in weeks 8, 9, and 10 were lower than levels measured in weeks 1, 3-6, 12, 13, 15, 16, 18 and 19.

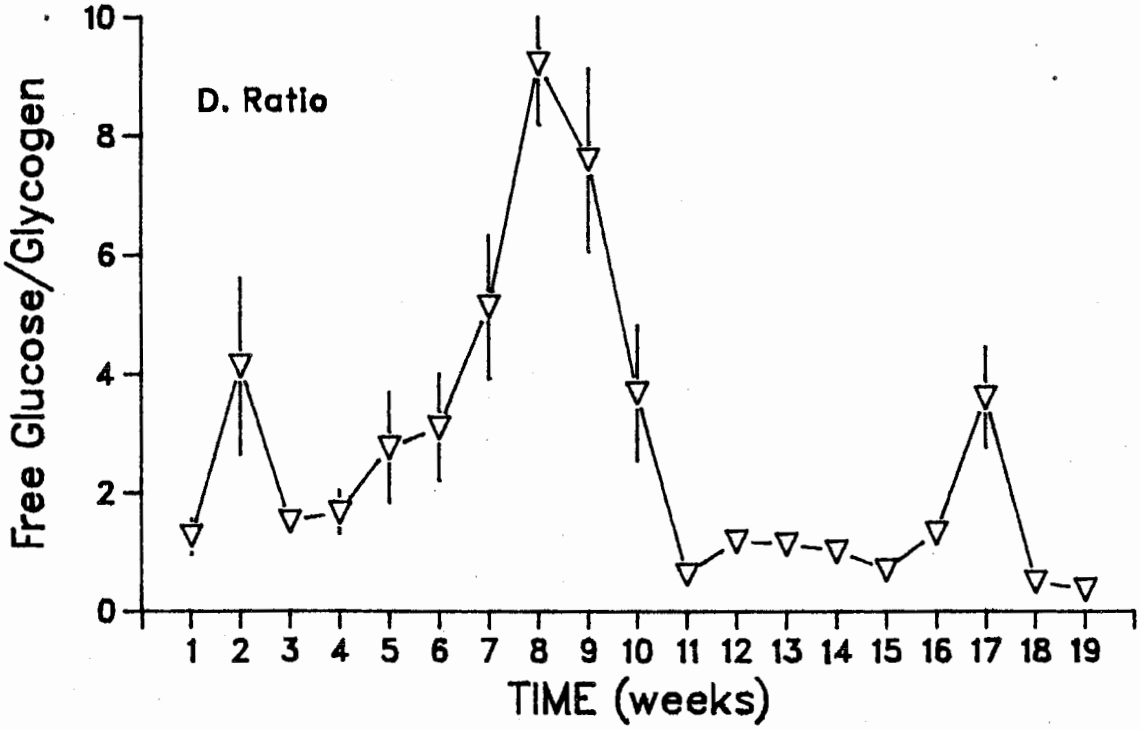
The second carbohydrate component of the liver tissue, that of free glucose, is shown in Figure 13c. As seen in the previous two plots, there was a significant depression in tissue concentration of this parameter during weeks 8, 9 and 10. Unlike the muscle and liver glycogen, however, there was no general decline and recovery observed in liver free glucose over the sampling period, and, in general, the concentrations of liver free glucose were more tightly maintained than liver glycogen. Statistically, fish from weeks 8, 9 and 10 had lower liver free glucose levels than those from all other weeks. As an indicator of carbohydrate metabolism in the liver, the free glucose/glycogen ratio is shown in Figure 13d. The profile for this parameter demonstrated a distinctive peak, centred at weeks 8 and 9. The ratios from these two weeks were higher than all other sampling weeks. Week 7, representing the initial rise in this peak, had a significantly higher free glucose/glycogen ratio than weeks 1, 3, 4, 11, 12, 13, 15, 16, 18 and 19.

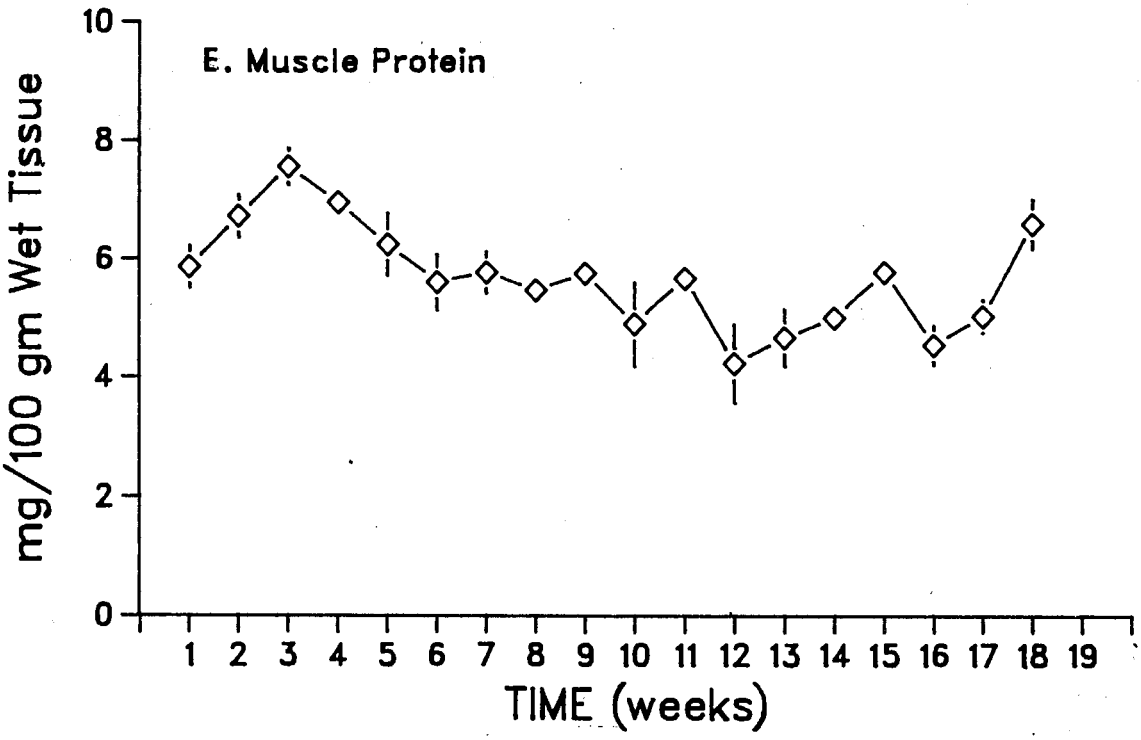
Figure 13:1983 coho smoltification study: tissue metabolite levels. (A) Muscle glycogen. (B) Liver glycogen. (C) Liver free glucose. (D)Ratio of free glucose to glycogen in the liver. (E) Muscle protein. (F)Muscle amino acid nitrogen. Each point represents the mean (n=8-10) ± S.E.M. All values, except those of (D) are expressed as mg/100 gm of wet tissue. See text for detailed discussion.

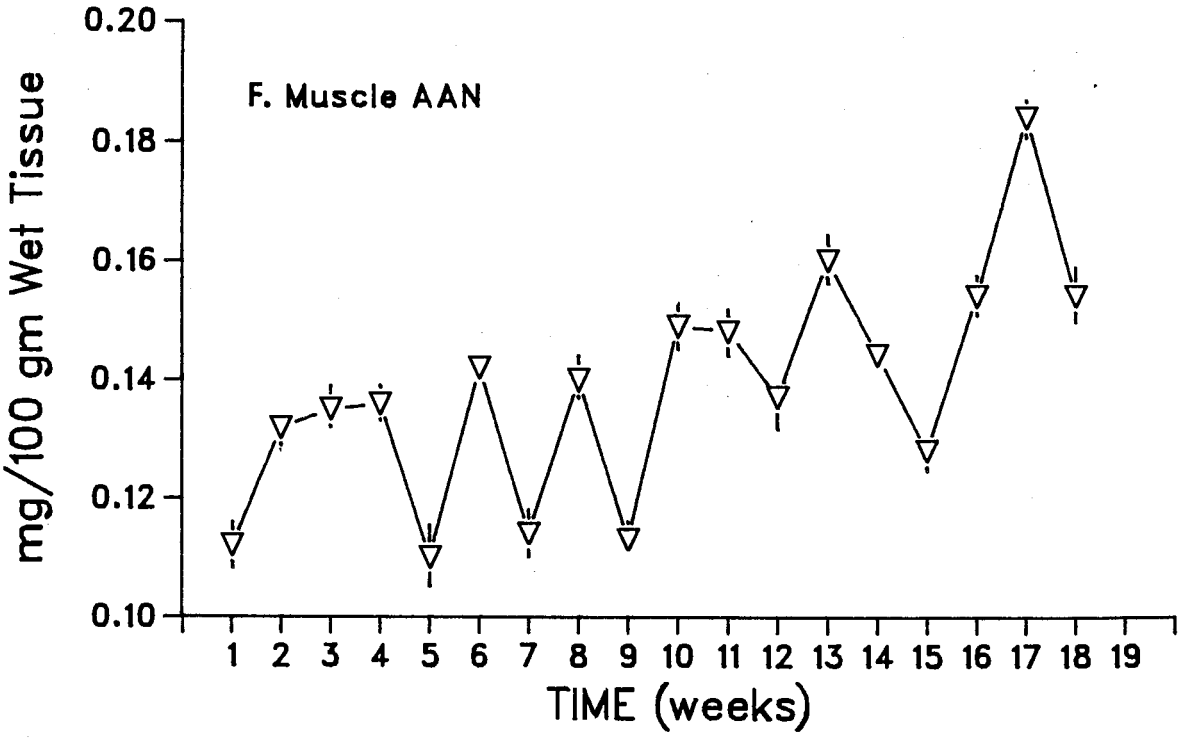

A. Muscle Glycogen
mg/gm Wet Tissue
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


B. Liver Glycogen
mg/gm Wet Tissue
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


C. Liver Free Glucose
mg/gm Wet Tissue
12
9
6
3
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


D. Ratio
Free Glucose/Glycogen
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


E. Muscle Protein
mg/100 gm Wet Tissue
0
2
4
6
8
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)


F. Muscle AAN
mg/100 gm Wet Tissue
0.10
0.12
0.14
0.16
0.18
0.20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TIME (weeks)

Muscle protein (Fig. 13e) concentrations were quite stable over the sampling regime, although the slight peak value achieved by the week 3 fish was higher than that seen in weeks 1, 6, 7, 8, 10, 12, 13, 16 and 17. Overall, this parameter had a slightly negative slope over the 19 weeks of sampling ($Y = -.0945X + 6.578$; $R^2=.105$;$P < .001$). Over the initial 7 weeks the regression was not as strong but the slope was even greater ($Y = -.13655X + 6.925$;$R^2=.0406$;$P < .05$). The amino acid content of the muscle tissue (mAAN) is plotted in Fig. 13f. This parameter demonstrated large weekly variations over the 19 sampling periods, although there was no indication of a lunar entrainment as seen in the plasma AAN and glucose patterns. The mAAN profile seemed to be characterised by declines rather than peaks, such that the minimal values observed in weeks 1, 5, 7 and 9 were lower than all other weeks sampled, whereas the peak value at week 17 was higher than all other means. As is obvious from the plot, this parameter had a positive slope over the 19 weeks ($Y = .00239X + .1158$;$R^2 = .3253$;$P < .001$). The regression over the initial 7 weeks was nonsignificant. Neither protein or AAN content in the muscle demonstrate any indication of the large depletion seen in the carbohydrate moiety of both muscle and liver tissues.

Table 3 displays the correlation matrix of these various parameters over the 19 weeks of this study. Both weight and length showed a positive correlation with plasma glucose but negative correlations with liver glycogen and muscle AAN. Weight, but not length, demonstrated a negative correlation with plasma GH levels. Weight also demonstrated strong positive correlations with muscle protein and muscle glycogen. However, it was length and not weight that demonstrated correlations with the lipid components. Length and the total FFA concentration showed a negative correlation, due primarily to the even stronger correlation seen between length and the level of plasma unsaturated FFA. The correlation between length and saturated FFA

Table 3.: Correlations between parameters of the 1983 sampling period. The abbreviations are as described in the text. The correlations are one-tailed where single stars (*) represent ($P \leq .01$) and double stars (**) indicate ($P \leq .001$). See text for detailed discussion.

	Weight	Length	Cond. Factor	Hemato	Plasma GH	Plasma AAN	Plasma Glucose	Muscle Protein	Muscle AAN
Weight	1.0000	.8341**	-.0732	.0842	-.2175*	.0490	.2396**	.3255**	-.4478**
Length		1.0000	-.5746**	.0602	-.0046	-.1245	.1943	-.0293	-.4526**
C. Fact.			1.0000	-.0298	-.3686**	.2857**	-.0075	.5516**	.0335
Hemato				1.0000	-.1043	.0913	.1539	.2656**	.0067
GH					1.0000	-.2422**	-.1857*	-.4707**	.3943**
P-AAN						1.0000	.2285*	.1063	.0076
P-Glucose							1.0000	.1690	-.1906*
M-Protein								1.0000	-.1739
M-AAN									1.0000

	Muscle Glycogen	Liver Glycogen	Liver Glucose	Ratio Glu/Gly	Plasma satFFA	Plasma unsatFFA	Plasma Total FFA	Ratio sat/unsat
Weight	.2029*	-.1978*	.1658	.0032	-.1586	-.0860	-.1482	-.1823
Length	.0583	-.3908**	.0734	.0745	-.2029	-.2543*	-.2378*	.0314
C. Fact.	.2399**	.4276**	.3642**	-.1629	.0943	.3075**	.1738	-.3793**
Hemato	-.1397	-.1224	.1375	.1290	.1020	.1846	.1385	-.1938
GH	-.2004*	-.2363*	-.2737**	.0362	.2528**	.0744	.2120	.4099**
P-AAN	.2485**	.3801**	.3280**	-.2759**	-.2204**	-.0679	-.1893	-.1699
P-Glucose	.1390	.0825	.2208*	.0768	-.1482	-.1812	-.1723	.0056
M-Protein	.1983*	.1931*	.4534**	-.0338	-.1031	.1524	-.0222	-.4182**
M-AAN	-.1970*	.0949	.0615	-.0781	.3185**	.3280**	.3497**	-.0308
M-Glycogen	1.0000	.3316**	.3672**	-.2645**	-.0199	-.0751	-.0413	-.0490
L-Glycogen		1.0000	.5033**	-.5798**	.1152	.2348*	.1671	-.2341*
L-Glucose			1.0000	-.4910**	.2257*	.2934**	.2650*	-.3188**
Ratio				1.0000	-.1494	-.1128	-.1498	.0549
satFFA					1.0000	.6528**	.9696**	.1643
unsatFFA						1.0000	.8183**	-.4553**
Total FFA							1.0000	-.0224
Ratio S/U								1.0000

was not significant (P = .013). Condition factor, an index of weight:length relationships during growth, showed a negative correlation with plasma GH concentrations but very strong positive correlations with muscle protein, liver glycogen, liver free glucose, plasma unsaturated FFA, plasma AAN and muscle glycogen, all of which were highly significant ($P \leq .001$).

In addition to its relationship with weight and condition factor, plasma GH levels showed very strong but opposite correlations with muscle protein ($r = -.4707$) and muscle amino acid nitrogen ($r = .3943$). On the other hand, plasma GH and plasma amino acid nitrogen had a negative correlation as did plasma GH with liver free glucose, liver glycogen and muscle glycogen. Positive correlations were observed between plasma GH and plasma saturated FFA and total FFA concentrations.

Amongst the other plasma parameters, plasma AAN showed highly significant positive correlations with liver glycogen, liver free glucose and muscle glycogen, but demonstrated a negative correlation with plasma saturated FFA. The correlation to both liver glycogen and liver free glucose further led to a strong and negative correlation between plasma AAN and the ratio of these two hepatic parameters. The correlation between plasma AAN and plasma glucose was also significant. However, plasma glucose showed little correlation with tissue parameters, the strongest being with the free glucose level in the liver.

Within the tissue compartments, liver glycogen and liver free glucose showed a strong positive correlation, as one might expect. Liver glycogen also showed a positive correlation to muscle glycogen and muscle protein parameters. Interestingly, liver glycogen demonstrated a strong correlation with plasma unsaturated FFA but not with saturated FFA (P = .115). As well as having a strong correlation with liver glycogen , muscle glycogen demonstrated a strong correlation with the free glucose content

of hepatic tissue. Muscle glycogen also showed weak but significant ($P \leq .01$) correlations with both protein and AAN content in the muscle while there was a very strong positive correlation between muscle protein and liver free glucose titers The muscle AAN correlations with plasma total FFA, saturated FFA and unsaturated FFA were all very highly significant ($P \leq .001$).

Since there seemed to be some indication of a difference between pre-release fish (week 1-7) and post-release fish (week 8-19), Table 4 shows the correlations between the parameters over the initial 7 weeks of sampling. Over this time period, weight and length no longer showed correlations with plasma glucose. In contrast, weight showed a strong but now positive correlation with liver glycogen and liver free glucose. Weight did, however, have the same strong correlation with muscle protein levels as it did over the total 19 weeks. Condition factor again exhibited a negative correlation with plasma GH as seen in the previous table. Condition factor also maintained similar correlations with muscle protein, liver glycogen and liver free glucose. There were, however, no longer any correlations with plasma AAN or muscle glycogen levels. It seems that although weight and length alone did not maintain correlations with the various parameters over the sampling regime, the overall growth pattern was very stable with regards to certain parameters, especially tissue stores.

Other than its correlation with condition factor, plasma GH showed no significant correlations with any parameters during weeks 1-7. Plasma AAN levels, on the other hand, demonstrated very strong negative correlations with both total FFA and saturated FFA concentrations over these 7 weeks. Plasma AAN levels and the liver and muscle glycogen levels over 19 weeks were highly significantly correlated (Table 3). In the initial 7 weeks, these correlations were not quite significant (with liver glycogen, $P = .011$;with muscle glycogen, $P = .012$) but the

Table 4.: Correlations between parameters in the initial 7 weeks of the sampling period. The abbreviations are as described in the text. The correlations are one-tailed where single stars (*) represent ($P \leq .01$) and double stars (**) indicate ($P \leq .001$). See text for detailed discussion.

	Weight	Length	Cond. Factor	Hemato	Plasma GH	Plasma AAN	Plasma Glucose	Muscle Protein	Muscle AAN
Weight	1.0000	.8091**	-.0032	-.0629	.0857	.1551	.1831	.3397*	-.0378
Length		1.0000	-.5811**	.0862	.2537	.0511	.2709	.0068	-.1057
C. Fact.			1.0000	.0814	-.3187*	.1174	-.1868	.4460**	.1245
Hemato				1.0000	-.0292	.1393	-.0729	.0727	-.1293
GH					1.0000	-.0765	-.1211	-.1351	.1574
P-AAN						1.0000	.0645	-.1217	.0264
P-Glucose							1.0000	.0371	-.3085*
M-Protein								1.0000	.1547
M-AAN									1.0000

	Muscle Glycogen	Liver Glycogen	Liver Glucose	Ratio Glu/Gly	Plasma satFFA	Plasma unsatFFA	Plasma Total FFA	Ratio sat/unsat
Weight	.2303	.4327**	.4945**	-.2771	-.0062	.0344	.0113	-.2543
Length	.0568	.1338	.2095	.0784	.0138	.0963	-.0306	-.0168
C. Fact.	.1642	.3943**	.3515*	-.2897*	-.0370	.2147	.0719	-.3197
Hemato	-.0283	.0857	.2134	-.0128	.0228	.0169	.0219	-.0553
GH	-.1708	-.2230	-.1440	.2532	-.1492	-.2314	-.1967	.2109
P-AAN	.2763	.2758	.1543	-.1882	-.4110**	-.1570	-.3290**	-.0892
P-Glucose	.0802	-.0824	.0309	.0876	-.0369	-.1763	-.1022	.1664
M-Protein	.1300	.4320**	.6084**	-.3245*	.0455	.2895*	.1571	-.5334**
M-AAN	-.0884	.0133	.0989	.0352	.0197	.1901	.0965	.1879
M-Glycogen	1.0000	.5871**	.3747*	-.3085*	-.0172	-.0333	-.0255	-.1074
L-Glycogen		1.0000	.5667**	-.7231**	.0171	.1980	.0986	-.3798*
L-Glucose			1.0000	-.3676*	.2359	.2759	.2710	-.3625*
Ratio Glu/Gly				1.0000	.1691	-.0182	.1037	.2614
satFFA					1.0000	.7234**	.9590**	-.2317
unsatFFA						1.0000	.8894**	-.7343**
Total FFA							1.0000	-.4547**
Ratio S/U								1.0000

tendencies suggest a similar relationship between these variables. However, there were no such indications between plasma AAN and liver free glucose. Plasma glucose and muscle AAN, on the other hand, did exhibit a strong negative correlation over 7 weeks.

Unlike the plasma parameters, correlations between tissue levels of carbohydrates and proteins over weeks 1-7 were almost identical to the correlations seen over the entire 19 weeks. Liver glycogen again demonstrated very strong positive correlations between liver free glucose, muscle glycogen and muscle protein. Muscle glycogen maintained a strong positive correlation with liver free glucose but no longer exhibited correlations with muscle protein or mAAN. Recall that these last two correlations were weak over the 19 weeks as well. Finally, muscle protein again had a very strong positive correlation with liver free glucose. There were no significant correlations between any of the tissue parameters and the levels of FFA in the plasma, even with the mAAN component which was so significant over 19 weeks.

DISCUSSION

The injection experiments presented several interesting results. Firstly, both salmon and ovine GH elicited similar responses: both caused hyperglycemia in juvenile coho salmon and both had little effect on plasma or tissue titers of protein or lipids. The similarity in degree of response suggested that ovine GH may be approximately equipotent, at least in terms of hyperglycemia. The efficacy of mammalian GH has been demonstrated many times in the literature as a promoter of growth in young salmon, although bovine GH does not always seem to be as effective as other mammalian GHs (Higgs *et al.*, 1975, 1976, 1977;Komourdjian *et al.*, 1976), possibly due to its dimeric configuration. Clarke *et al.* (1977) also reported on the efficacy of teleost GH in promoting growth in juvenile sockeye salmon. Additionally, several authors have demonstrated the nitrogen retaining effects of GHs in fish (Matty, 1962;Higgs *et al.*, 1975, 1976;Smith and Thorpe, 1976). In view of the relative success of GH treatment in promoting growth in fish in previous studies, it was somewhat surprising that neither GH was effective in altering plasma or tissue AAN or protein levels in the present study. Most of these experiments, however, were of considerably longer duration than were my injection experiments, with treatments lasting weeks and months instead of 24-48 hours. Further, previous studies have repeatably demonstrated that endocrine effects, particularly those of peptide hormones, are slower to develop in fish, often requiring hours or days to elicit effects that are observed in minutes to hours in higher vertebrates. This retardation of response noted in teleosts may, in part, be due to the much lower metabolic rates. In agreement with my results, Inui and Ishioka (1985) also noted an appreciable lag in GH-mediated effects on protein metabolism in juvenile masu salmon. Thus, the lack of effect of either of the GHs on protein or lipid metabolism in my injection experiments

may have been due to inappropriate sampling regimes rather than to a real lack of effect. It was very unfortunate that these experiments could not be continued due to limitations in salmon GH available.

The second interesting result from the injection experiments was the total lack of response of either GH treatment observed in the third injection series, ie., that of June 20 when the fish may be considered to be fully smoltified. This lack of response is particularly interesting in view of the greater than 2-fold hyperglycemia observed in the previous two injection series. These results seem to indicate that, early in the parr-smolt transformation, the fish are responsive to GH whereas the fully smolted fish appear to be refractory. Interestingly, similar effects were noted by Sheridan (1986). He reported that *in vivo* treatment of juvenile coho parr with bGH, oPRL, T_4 or cortisol greatly enhanced lipid mobilisation (via enhanced triacylglycerol lipase activity). In smolts, however, these responses were not observed. Further, the level of lipase activity in tissues of non-treated smolts was considerably elevated over that observed in the tissues of the non-treated parrs. Sheridan suggested that the results were indications that the metabolism and the GH-mediated responses were already at their highest effective rate and, therefore, increasing the circulating concentration of GH was unable to increase these rates any further. However, a second mechanism is also a possible mediator of this anomaly, and that is the down-regulation of the receptors or a decrease in receptor affinity during the course of smoltification. Increased circulatory levels of protein hormones, particularly if the hypersecretion is chronic, will often lead to a decrease in tissue sensitivity and responsiveness (for review, see Jänne and Kontula, 1980).

In partial support of both these suggestions, tissue and plasma levels of glucose, AAN, protein and FFAs all declined over

the experimental period of these injection studies (ie, February to June), which may be considered equivalent to the smoltification period. As will be discussed shortly, depletion of body energy stores is characteristic of the parr-smolt transformation. If such stores are indeed depleted by the end of smoltification, then it is possible that increased circulatory levels of GH might be unable to elicit further response. Further, since there seemed to be relatively large increases in circulating levels of GH during the parr-smolt process (from the weekly sampling studies), the increased mobilization of body lipid, carbohydrate and protein stores was presumably the partial result of increased hormone activity. These increased circulatory levels of GH may induce changes in tissue responsiveness. Changes in receptor numbers would indicate altered rates of uptake by the tissues, whereas changes in receptor affinities would not require such an increase. Additionally, receptor affinities or numbers or the post-receptor responses may be mediated by other hormonal factors (eg., cortisol, insulin, T_4/T_3, somatostatin) which also change in plasma concentration over the smolt transition. A number of studies in mammalian systems have demonstrated that tissue responsiveness to growth hormone(s) can be very dependent upon circulating levels of these and other hormones (Bauman *et al.*, 1982). As well as endogenous conditions, the possible influence of lunar (or other exogenous) rhythms over these aspects is also important. The interrelationships of these possible scenarios cannot be answered from the present research and will be the focus of my future studies.

The parr-smolt transformation is characterized by a large increase in specific growth rate, over and above the enhanced growth seen in most temperate zone fish in the early spring and summer (reviewed by Wedemeyer *et al.*, 1980). While increased somatotroph activity (and, thus, elevated GH secretion) have been shown to be strongly correlated with enhanced growth rates (eg.,

Swift and Pickford, 1962, 1965;reviewed by Donaldson *et al.*, 1979), increased plasma titers of GH may also be directed to metabolic pathways not associated with growth. For example, stress, exercise and starvation all stimulate release of GH from the pituitary in mammals, even though these situations are usually periods of little or no growth. Exercise and starvation have also been shown to elevate plasma GH levels in salmonids (Wagner, 1985;Barrett and McKeown, 1988) whereas stress does not seem to elicit such a response (Wagner, 1985;A.D. Pickering, pers. comm.). These increases in plasma GH may serve to increase mobilisation and plasma levels of energy-rich compounds which may be utilised by the fish to stimulate metabolism without stimulating growth.

The increase in circulating GH levels during smoltification in both study years strongly supports the proposition that GH mediates aspects of the parr-smolt transformation. These long-term elevations also corroborate the histological evidence of increased synthetic and secretroy activities of the pituirary somatotroph cells, as noted by several authors (Olivereau, 1954;Clarke and Nagahama, 1977;Nishioka *et al.*, 1982). Further, the association of the elevated plasma GH with the latter stages of smoltification is very similar to changes in the other two major hormones which have been associated with smoltification of salmonids, namely thyroxine (Dickhoff *et al.*, 1978, 1982;Grau *et al.*, 1982;Youngson and Simpson, 1985;Dickhoff and Sullivan, 1987) and cortisol (Specker and Schreck, 1982;Barton *et al.*, 1985;Young, 1985) (reviewed by Wedemeyer *et al.*, 1980;Nishioka *et al.*, 1982;McKeown, 1984;Barron, 1986). Both the thyroid gland and the adrenal gland show increased activity over the parr-smolt transformation and large increases in plasma thyroid and cortisol hormone concentration(s) in late smolts. Specker *et al.* (1984) suggested that some aspects of the surges in plasma T_4 may be mediated by changes in target tissue uptake of this hormone, as

discussed earlier for GH. Numerous studies have demonstrated that the synergism existing between GH and T_4 seen in mammals very probably also exists in teleosts (reviewed by Donaldson *et al.* 1979;Leatherland *et al.* , 1982), including the monodeiodination of T_4 to T_3 by GH, as suggested by the work of DeLuze and Leloup (1984), Miwa and Inui (1985). Milne and Leatherland (1978) reported that injections of bovine GH led to significant increases in plasma T_4 and T_3 concentrations in rainbow trout. Further, Leatherland and Hyder (1975) demonstrated that exogenous thyroxine enhanced somatotroph activity (increased endoplasmic reticulum, larger mitochondria, enhanced granule secretion) in juvenile *Tilapia zillii* as compared to controls. Cortisol has also been shown to act on many of the same tissues as GH and T_4 in teleosts, although often in opposite directions (reviewed by Donaldson *et al.* , 1979;Higgs *et al.* , 1982; Barron, 1986). Björnsson *et al.* (1987) further examined the relationships between these three hormones in hypophysectomized coho smolts and reported many interactive effects on growth as well as on aspects of smoltification (eg., osmoregulation). The interplay of these and other hormones during the transition from parr to smolt are likely the cause of the variable patterns seen in plasma metabolite levels. Tissue receptor affinities as well as post-receptor responses are constantly under the influence of both positive and negative modulators, the sum of which can be seen as changes in metabolite levels at both the tissue and plasma level.

The fish undergoing desmoltification exhibited continuing and increasing elevations in plasma GH. These fish, presumably having missed the ideal migration period, are undergoing reversion to freshwater parr conditions, as seen by the return of many tissue parameters to pre-smolt levels. The transfer of these remaining fish to smaller holding tanks over the course of the summer months may have had some effect on plasma GH levels, although

stress does not induce GH elevations in salmon. However, these fish, under natural conditions, would probably be experiencing increased growth rates, as resources previously diverted to smoltification are re-mobilized and available. Additionally, water temperatures are increasing over this time period, surpassing 10°C for the first time in early August, ie., at a time when GH levels exhibited a very large increase. Studies have shown that the optimal rearing temperature, in terms of growth rate, is between 10-12°C for salmon (eg., Atherton and Aitken, 1970). Work by Swift (1954), Swift and Pickford (1962, 1965) demonstrated that maximal GH secretion in teleosts occurs in the summer months.a Thus, these fish are experiencing high temperatures, a possible excess of anabolic precursors, and minimal competition for additional resources (food). While they were smaller in mean size, these fish may be experiencing quite rapid individual metabolic and growth rates. However, the rate of plasma GH increase was far greater in the smolting fish than in those fish retained in freshwater.

The wide variation in plasma glucose concentrations in both of the weekly sampling years is presumably a reflection of the changes in metabolism occurring over the smoltification months. Generally speaking, the levels seemed to be increasing as smoltification proceeded. Increased levels of plasma glucose in smolts versus parrs was also reported by Woo *et al*. (1978), although the authors did not measure weekly variations. It is difficult to ascertain whether the dramatic peaks and declines exhibited by circulatory glucose are the result of increases/decreases in uptake or in secretion. It is probably a highly complex interaction of both actions. Certain tissues may, at certain times, require high levels of glucose for metabolic purposes, whereas at other times the demand will be reduced. The somewhat cyclical nature of the plasma glucose titers (as well as the circulatory amino acid nitrogen levels) may be mediated by

lunar rhythms. The 1982 morphological data, particularly the weight, demonstrated a non-linear somewhat cyclical increase over the 4 month sampling period. The values for weight appeared to peak approximately 4 weeks apart. When plotted against the lunar calendar these peaks were within a week of the new moon phase. When plasma glucose and amino acid nitrogen values were analysed, a similar cyclical pattern emerged. Plotting these parameters along a lunar calendar, the glucose values peak around full moons, whereas the plasma amino acid nitrogen values, rather than displaying obvious peaks, appear to have their lowest values around new moons. Plotting the "rate" of weight increase against plasma glucose values, the two cycle in opposition to each other. This data suggests that at different times during the smolt transformation the fish were in different metabolic states and that these states were mediated by lunar rhythms. The morphological data for the 1983 sampling experiment do not exhibit this lunar cycle. However, plasma glucose and AAN demonstrated strong cyclical patterns, both parameters appearing to peak around full moons. This pattern appears strongest around the release date and seems to diminish and possibly shift ahead slightly as the summer solstice is passed and the fish revert to parr. When growth seemed to be greatest, plasma amino acid nitrogen levels were at their lowest values. When growth seemed to be at lower rates, plasma glucose appeared at peak values. While these observations are only the result of serial samples from the population and , therefore, may be correlated by chance, a possible hypothesis does emerge: if there are periods within the smolt 'window' when outmigration is optimal, then it would be advantageous for the smolts to be behaviourally and energetically prepared (eg, increased mobilization of energy supplies). At periods when outmigrations are not optimal, then growth may take precedence. In early smoltification and late desmoltification, such cycling may not be apparent as the fish are not physiologically adapted for a marine environment, and

outmigration is not an option the parr possess. Lunar cycles as an exogenous *zeitgeber* have been proposed for behavioural (Mason, 1975;Grau *et al.*, 1981;Youngson *et al.*, 1983) as well as physiological or, ultimately, endocrinological patterns of smoltification (Grau *et al.*, 1981,1982;Farbridge and Leatherland, 1987), but has yet to be definitively proven. Lunar cycles may provide the premigrant but anticipatory smolts with proximal cues to enhance successful migration and/or ocean entry (eg., high tides, marine plankton cycles, etc.). Variations in nutrition, temperature, migration distance, etc. could obscure or alter such cycles in different populations or within the same population in different years. However interesting, the data is suggestive only and this 'model' remains purely speculative.

Although plasma GH and plasma glucose levels were significantly correlated in the 1983 study, the non-significant correlation in 1982 possessed an almost identical R value, suggesting a very weak relationship between the two parameters, However, hormones tend to act more directly on metabolite turnover rates than upon storage levels (Murat *et al.*, 1981) and that changes in rates of secretion are often coupled or preceded by changes in rates of uptake. Additionally, the well documented changes in plasma levels of cortisol, T_4, etc. may alter any direct relationships. Thus, one cannot always expect to observe that endogenous levels of one of a number of hormones controlling metabolism will be a direct reflection of circulatory levels of metabolites.

It was obvious, however, that, as GH levels increased over smoltification, body glycogen levels were rapidly declining. This decline in body glycogen stores, observed in the 1983 fish as smoltification proceeded, was in agreement with earlier reports (Fontaine and Hatey, 1950; Wendt and Saunders, 1973;Woo *et al.*, 1978). This decline is apparently an important characteristic of the parr-smolt transformation. In terrestrial vertebrates,

carbohydrates (along with lipids) are the main source of oxidative energy production, whereas in teleosts, proteins and lipids are primarily utilized. Starvation of higher vertebrates can easily exhaust liver glycogen stores within 24-48 hours, whereas in a number of teleosts, many studies have shown that long-term starvation, even in excess of 3-6 months, does not exhaust and, in some cases, has led to increased liver glycogen content (McKeown, 1984;Weatherly and Gill, 1987). Additionally, fish exhibit poor regulatory capacity for glucose, unlike higher vertebrates (except birds and ruminants). Injection of glucose into most vertebrates leads to transient hyperglycemia followed by a rapid return to homeostatic plasma levels. Fish, on the other hand, demonstrate a diabetic type of response to glucose injection: prolonged hyperglycemia and associated glycosuria. This lack of importance of carbohydrate (relative to proteins and fats) in fish is presumably a reflection of dietary sources in the wild. Whereas hatchery-fed fish may encounter carbohydrate overloads, the diet of wild populations is mainly insect and crustaceans, ie., mainly proteins and lipids (Cowey and Sargent, 1977;Walton and Cowey, 1982). In fact, studies have shown that there is no essential requirement for dietary carbohydrates by fish (Weatherly and Gill, 1987). Additionally, hormones (eg., insulin) which, in higher vertebrates have important regulatory actions on carbohydrate homeostasis often have greater importance in protein or fat metabolism or both in fish (Plisetskaya, 1980;Walton and Cowey, 1982;Ince, 1983;Plisetskaya *et al.*, 1986;Weatherly and Gill, 1987). Adult coho salmon have been reported to increase hepatic glycogen levels during upstream migration, a period of intense exercise and no caloric intake (McKeown, 1984). Long-term starvation in eels, carp and other teleosts has also led to increased or stable liver glycogen titers (reviewed by Cowey and Sargent, 1979). Thus, the decline of tissue concentrations of this compound during smoltification suggests either an extremely active metabolism which lipid and/or

protein catabolism cannot sufficiently supply fuels for, or an enhanced carbohydrate metabolism directed in a specific pathway essential for successful smoltification, such as enhanced production of the glycoproteinaceous Na^+,K^+-ATPase enzyme. The coincident depletions of and high correlations between muscle glycogen and both liver glycogen and liver free glucose levels over the initial 7 weeks of the 1983 sampling demonstrate a general depletion of body carbohydrate stores. Sheridan *et al.* (1985b) have demonstrated that the depletion of hepatic glycogen stores during smoltification is associated with increases in glycogenolytic enzyme (glycogen phosphorylase *a*) activity as well as decreases in the activity of glycogen synthetase. While GH is known to enhance hepatic glycogenolysis in higher vertebrates (ie., a diabetogenic effect), such a relationship has yet to be firmly established in fish. The hyperglycemic response noted in the parr of the first injection experiment does intimate that GH may exert these effects in fish as well, although the lack of correlation between plasma GH titers and either liver glycogen or liver glucose over the initial 7 weeks of the 1983 weekly sampling study does not seem to support this interaction. However, when the data was analyzed on a body weight basis, a very strong ($P < .001$) negative correlation between plasma GH and liver glycogen levels ($r=-.4034;n=68$) was noted (data not shown). Additionally, in the initial 7 weeks, when tissue glycogen and glucose levels were declining, the regression slope for plasma GH was 4.74, whereas over the subsequent 11 weeks, as carbohydrate stores returned to parr-like levels, the slope for plasma GH was barely half of this value: 2.76. Examination of muscle glycogen or liver free glucose and plasma GH levels on a per weight basis did not yield any significant correlations. Receptors for GH have been reported to exist on teleost hepatic microsomal membranes (Fryer, 1979), as in higher vertebrates. Furthermore, seawater 'stunts' (young salmon able to survive but not grow in seawater) demonstrate decreased specific binding of GH in hepatic (as well

as in gill and kidney) tissue (Fryer and Bern, 1979), coupled with a lack of hypoglycemia as seen in normal smolts in seawater (Woo *et al.*, 1978;Sweeting and McKeown, 1987). Plasma and pituitary GH levels in stunts, on the other hand, appear normal or even elevated over smolt levels (Clarke and Nagahama, 1977;Bolton *et al.*, 1987b). This provides further indications of a link between GH and carbohydrate metabolism in teleosts. The inability of hepatic tissues to bind GH (due to a lack of receptors) is also seen in human systems, resulting in Laron dwarfism. Thus, mediation of hepatic glycogenolysis by changes in plasma GH may be an important feature of successful smoltification. Interestingly, Fryer (1979) was unable to demonstrate specific (displaceable) binding of GH to muscle, suggesting either no receptor or one of extremely low affinity. It is very possible that muscle glycogen metabolism may be mediated by other hormones, singularly or in conjunction with GH. It seems very unlikely, however, that muscle cells possess no receptors for GH, as this tissue is the primary growth tissue. Decreases in liver glucose concentrations, which were also seen during smoltification, suggests that the carbohydrate moiety was either being diverted to alternate pathways, ie. conversion to lipid or protein within the hepatic tissue, or was being transported to extrahepatic sites. Plasma glucose levels were very high both immediately before and after this 3 week period, but exhibited low values during the time in question. This in itself does not answer the above question, as the low circulating levels could be further proof of increased extra-hepatic uptake or utilization. The muscle glycogen levels also exhibited a nadir over these three weeks. Again, the data suggest the smolts are in a high state of metabolism.

The 1983 study also examined these aspects of carbohydrate metabolism as the fish desmolted, ie., as they returned to parr-like parameters. As mentioned previously, the level of GH in

the plasma continued to rise over this period, although at a much slower rate. Plasma glucose levels over these weeks tended to be relatively stable, whereas both liver and muscle compartments exhibited large increases in glycogen concentrations. Hepatic glucose also rapidly returned to the previous levels. These changes in carbohydrates eventually lead to a strong negative correlation between plasma GH and both liver glycogen and glucose, as well as with muscle glycogen, over the entire 19 weeks of the 1983 study. This replenishment of body glycogen stores to parr-like values during desmoltification has also been reported previously (Malikova, 1959;Woo *et al.*, 1978). The return to a freshwater metabolism as the summer proceeds is presumably a consequence of decreasing probability of successful migration to the sea. The role of the highly elevated GH levels in carbohydrate metabolism at this time is unknown, but, under natural conditions, the fish would likely be experiencing high growth rates over this time.

The stability of muscle protein concentrations over smoltification (and the subsequent desmoltification) were in direct contrast to the large depletions in tissue glycogen levels. This lack of protein catabolism has been previously reported in the literature. Fontaine and Marchelidon (1971) reported that Atlantic salmon smolts did not display increased utilization of muscle protein. Woo *et al.* (1978) also found no differences in liver or muscle protein content in coho or chinook salmon smolts as compared to either parr or post-smolts, but the authors did note lower serum protein levels in the smolts. Fessler and Wagner (1969) reported decreases in body protein in large (> 14cm) rainbow trout undergoing smoltification, whereas such a response was not observed in smaller fish. The stability of this parameter during smoltification and subsequent desmoltification indicates muscle protein is not being catabolised as energetic sources. Farmer *et al.* (1978), sampling

1+ or 2+ Atlantic salmon smolts on a variable bi-weekly basis, noted relatively stable body protein levels, with some fluctuations. These authors also suggested the maintenance of body protein (expressed as percent body weight) during the parr-smolt transformation was an indication that increased lipid (and perhaps, carbohydrate) mobilisation and oxidation provided sufficient energy for the increased metabolism observed over smoltification. The depletion of body glycogen reserves and enhanced lipolysis noted in my sampling studies supports this. The highly negative correlation between plasma GH and muscle protein was somewhat surprising, as the stimulatory actions of GH on both amino acid uptake and protein synthesis are well established in higher vertebrates (eg., Turner and Bagnara, 1976). Additionally, a number of studies utilizing both mammalian and teleost GH preparations have demonstrated growth-promoting activity. This negative correlation between plasma GH and muscle protein was primarily due to the large increases in circulating GH over the time course of the study. This correlation is not significant during the initial 7 weeks, although it was still negative. Further, this parameter is being expressed as mg protein per unit muscle, which will not indicate increased units of muscle, i.e., increases in overall muscle mass. The data does show, however, that there is no smoltification-associated decrease in muscle intracellular protein, despite the high catabolic metabolism seen in carbohydrates and lipids. Additionally, Plisetskaya *et al*. (1984) has shown that insulin may also play a large role in protein metabolism in fish. The metabolic actions of this hormone during either smoltification or seawater adaptation remain to be investigated.

In contrast to the maintenance of muscle protein content, levels of muscle amino acid nitrogen in the 1983 study demonstrated some large fluctuations, particularly prior to and immediately following release from the hatchery. These large

perturbations are also seen in the plasma levels of amino acid nitrogen in both of the study years. Several authors have noted changes in amino acid and other non-protein nitrogen concentrations in muscle tissue during smoltification. Thus, Cowey and Parry (1963) reported a large increase in non-proteinaceous nitrogen in muscle tissue of smolts as compared to parr. Further, they noted the majority of this increase was due to elevated tissue creatine concentrations, suggesting either increased dephosphorylation of phosphocreatine (by increased endergonic reactions), or increased metabolism of the specific amino acids of which creatine is an end-product (ie., glycine, arginine and methionine). The authors reported no changes in total amino acid nitrogen, but noted decreases in the free amino acid concentration (%) in smolt muscle as compared to parr. However, the only specific amino acid seen to decrease in the above study was taurine, a sulphonated product of cysteine metabolism. The results obtained in the present study were obtained by the ninhydrin reaction, and thus changes in specific amino acids are not detectable. Additionally, the observed increase in muscle total amino acid nitrogen concentration may include increases in creatine, as the ninhydrin will react with the free amino and carboxyl groups of this compound. Fontaine and Marchelidon (1971), working with Atlantic salmon smolts, noted a significant decrease in free amino acid concentrations of muscle tissue, due to large decreases in threonine, glycine, histidine and taurine, while at the same time there were no changes in total amino acid concentration. These results also suggest that there was a concomitant increase in some non-protein nitrogen compound during smoltification of Atlantic salmon, perhaps in the creatine component. Recall also that Woo *et al*. (1978) noted lower serum protein content in salmonid smolts than were observed in parrs. There are no other reports of circulatory amino acid levels during smoltification. In contrast to these reports, Malikova (1959) noted quantitative differences among amino acids

within the protein molecules of muscle tissue. Thus, as smoltification proceeded, protein levels of methionine, tryptophan and histidine increased whereas levels of cysteine, tyrosine and arginine decreased. The author suggested that the resulting qualitative changes in muscle proteins enabled new structural and physicochemical properties that were important to the developing smolts.

An increased energy demand for increased endergonic reactions with the general increased metabolism associated with smoltification (Baraduc and Fontaine, 1955; Saunders and Henderson, 1970;Komourdjian *et al.*, 1976;Higgins, 1985). Changes in plasma and muscle amino acid concentrations may, in part, reflect the increased growth rates noted in smolting salmon. Alternatively, increased muscle tissue titers of specific amino acids, such as taurine and glycine, have also been suggested to serve as osmoregulatory compounds following seawater entry (Jurss, 1979;Assem and Hanke, 1983;Jurss *et al.*, 1983). The increased plasma GH levels seen upon seawater entry of salmon smolts (Sweeting *et al.*, 1985; Sweeting and McKeown, 1987) may therefore act in an osmoregulatory action by increasing uptake and intracellular concentration of amino acids. It seems possible as well that the developing smolts, possibly under osmotic stress in freshwater (Simpson, 1985), may be already utilizing specific amino acids for this purpose. Unlike the protein component in muscle tissue, the values of muscle AAN were increased as the fish desmolted, perhaps indicative of the levels which would have been observed in parr or pre-smolts. This 'recovery' response was similar to that observed in the tissue glycogen concentrations. Although plasma AAN levels did not appear to 'recover' to a higher parr-like value in the 1983 sampling year, circulatory levels of AAN did seem to decrease somewhat in the 1982 sampling experiment as well as over the time period associated with the injection experiments done in 1982. Similarly, plasma glucose

levels over the desmoltification period did not reflect the increase in tissue glycogen levels. Thus, merely measuring the circulating levels of these two parameters does not always reflect what is occurring within the depots, or how hormone levels may be affecting these two compartments. It was very interesting, however, that over the 19 weeks of the 1983 study, the protein and carbohydrate parameters exhibited some very strong correlations, primarily positive. Muscle protein also showed very high correlations with hepatic glycogen and glucose over the initial 7 weeks of this 1983 study. These correlations suggest a high degree of coordination between tissue levels of these two body components. Unfortunately, we were unable to determine tissue levels of the next parameter examined, that of the lipids. The lack of a correlation between plasma GH and either circulatory or tissue AAN levels during smoltification agrees with the lack of response noted in the injection experiments. It is generally observed that there is a lag response between GH treatment and increased protein metabolism (eg, Inui and Ishioka, 1985;Ishioka *et al.*, 1985).

There has been a great deal of research devoted to lipid nutrition and metabolism in fish (for reviews, see Cowey and Sargent, 1977;Greene and Selivonchick, 1987). The lipid mobilizing actions of GH in salmon have been demonstrated a number of times in whole body (Clarke *et al.*, 1977), muscle tissue (Higgs *et al.*, 1975, 1976, 1977), hepatic and mesenteric tissues (Sheridan, 1986). GH has also been shown to elevate plasma FFA levels in fish (Minick and Chavin, 1970). Sheridan (1986) further showed that GH-enhanced lipolysis was mediated by increased triacylglycerol lipase activity in liver tissues, although the effects of GH on mesenteric and dark muscle enzyme activities were limited. These studies suggest a strong cause:effect relationship between elevated plasma GH levels (Sweeting *et al.*, 1985), elevated lipolytic enzyme activities

(Sheridan *et al.*, 1985b), and depleted lipid stores (Vanstone and Markert, 1968;Fessler and Wagner, 1969;Saddler *et al.*, 1972;Woo *et al.*, 1978;Sheridan *et al.*, 1983) observed during the parr-smolt transformation. Few studies, however, have examined changes in plasma free fatty acids over smoltification. Mobilization of lipid, primarily from visceral depots in teleosts, provide a highly efficient energy compound for oxidative metabolism. In addition, oxidation of lipids (fatty acids) acts as a protein sparing mechanism, allowing amino acids to be diverted to protein synthesis (Cowey and Sargent, 1977;Greene and Selivonchick, 1987). This may explain some of the particularly strong correlation seen between plasma AAN and plasma free fatty acid components (both saturated and unsaturated) observed both in the initial 7 weeks as well as over the entire 19 weeks of the 1983 experiment. Mobilization of lipids from the adipose depots results from enzymatic degradation of triglycerides, releasing glycerol and free fatty acids from the intracellular compartment to the circulatory system. The majority of these fatty acids are subsequently esterified to plasma protein complexes of varying sizes and affinities, and are thus transported as lipoproteins (as VLDLs, LDLs, HDLs, etc.). Although the quantitative proportion of free or non-esterified fatty acids transported in the plasma of teleosts is very small, qualitatively they represent a very dynamic and important component (Kayama and Iijima, 1976;Cowey and Sargent, 1977;Zammit and Newsholme, 1979;Plisetskaya, 1980). The high metabolic rates associated with the parr-smolt transformation suggest that the rate of fatty acid oxidation could be enhanced considerably. Additionally, fatty acids are important structural components of plasma membranes, acting to maintain membrane fluidity and to regulate plasma membrane enzyme activities (Bell *et al.*, 1986). There is some evidence that saturated and monounsaturated fatty acids may be preferentially utilized for oxidative purposes (Shul'man, 1974;Jezierska *et al.*, 1982), freeing the highly

unsaturated fatty acids for specific metabolic (ie., non-oxidative) pathways or structural use.

The increased plasma levels of free fatty acids, both the unsaturated and saturated forms, over the 1982 sampling year was a clear indication that lipid mobilisation was indeed occurring. With very few exceptions, every FFA assayed showed higher circulating levels in smolts than in the parrs or pre-smolts. However, as with circulating titers of glucose and pAAN, there was no correlation between plasma FFAs and GH. Again, this lack of correlation may in itself be unimportant, as the hormone will be acting at the depot levels as shown by Sheridan (1986). There were, however, some interesting correlations observed. The very strong correlations observed between various plasma FFA components and condition factor confirm published reports: much of the decline in condition factor is attributed to decreases in the lipid contribution to weight of the animal. Thus, as lipid depots are depleted (and plasma FFA levels increase), condition factor also falls, leading to a negative correlation between condition factor and circulating FFA levels. Further, immediately following the large increases in plasma FFA levels seen in week 8, the rate of the decrease in condition factor was also seen to increase. The equally strong but positive correlation of plasma FFAs with hematocrit was, however, more puzzling. Hematocrits have been reported to increase over smoltification (Miles and Smith, 1968), although this was not particularly obvious in the 1983 study. I am unaware of any reports on interrelationships between lipid and RBC levels. There have been reports of alterations in the hemoglobin patterns as salmon smoltify (Vanstone *et al.*, 1964;Giles and Vanstone, 1976b;Koch, 1982;Sullivan *et al.*, 1985). This acquirement of adult forms of hemoglobin may be linked to changes in environmental parameters (O_2 levels, temperature, pH, etc.) that the migrating fish will face (Sullivan *et al.*, 1985) or to the increased metabolism and

oxygen requirements of the smolts as compared to the parr (cf. McCormick and Saunders, 1987). Plasma FFAs may be important either as energy sources or as structural components of RBC membranes or membrane enzyme systems (Bell *et al.* , 1986). The significance of this relationship requires further study.

In the 1983 study, the pre-release smolting fish demonstrated general declines in plasma levels of many of the individual saturated and unsaturated FFAs, although many (but not all) FFAs also demonstrated a small but significant peak value in the second week of sampling. This result was quite different from the one observed over smoltification in 1982. Some of these differences may arise from the later sampling date of this second study, ie., the plasma levels may already be high in this study in the initial weeks of sampling. However, a comparison of the two years shows that the levels of both saturated and unsaturated FFAs are considerably lower to corresponding periods in the 1983 study than in 1982. While different genetic, environmental and nutritional backgrounds limit the significance of such comparisons, some inferences may be made. The 1983 fish in the initial 7 weeks (ie., the pre-release smolts) had condition factors which were considerably lower than those observed in the corresponding weeks of the previous year, hinting that the 1983 fish may have already utilized much of their lipid depots prior to the onset of sampling. In the 1982 year, condition factor was strongly and inversely correlated with plasma saturated and total FFA levels, as well as with the sat/unsat ratio in the plasma. In the inital 7 weeks of the 1983 study, there were no significant correlations between condition factor and any of the FFA parameters. However, some interesting trends (which became significant over the entire 19 weeks) were apparent: condition factor again shows a high correlation to the plasma ratio of sat/unsat FFA, but there was no indication whatsoever of any such correlation to either saturated or total FFA in the plasma.

Instead, condition factor in these 1983 smolts demonstrated the highest correlation value to the unsaturated FFAs. It appears, then, that there was a large difference in the lipid metabolism patterns between the two years of study. Further, while plasma levels of glucose, AAN and GH were approximately equal for the corresponding weeks of the two study years, the possible early depletion of lipid stores in the 1983 fish may, in turn, have had an influence on the dramatic decrease in tissue carbohydrates observed over the late smoltification period in these fish (ie, weeks 6-9). Thus, the lower circulating levels of FFA seen in the 1983 fish may reflect an early depletion of lipid stores, necessitating an increased utilization of carbohydrates. A nearly complete utilisation of body lipids was also reported by Malikova (1959) in Baltic salmon, who stated that the period of most intense mobilisation occurred late in transition "during the period of most intensive smoltification". Again, differences in genetic, nutritional and environmental backgrounds make such comparisons difficult, even within the same hatchery.

As seen with the tissue glycogen levels, desmoltification is characterised by return of tissue lipids to parr values. The high plasma levels of FFA seen as the 1983 smolts reverted back to parr-like condition was also noted by Woo *et al*. (1978), who measured serum total lipids. These authors also reported greatly increased liver and muscle fat content in the desmolted fish as compared to the smolts. A return of body lipid levels to pre-smolt values was also reported by Malikova (1959). Sheridan *et al*. (1985b) further noted a decrease in triacylglycerol lipase activity as coho salmon smolts began desmoltification, leading to a decrease in the rate of lipid catabolism. Thus, the data from the two weekly sampling studies supports the prevailing theories: increased mobilization of tissue lipid during smoltification and increased lipogenesis and deposition as the fish desmoltify. The coincident high levels of GH and plasma FFA levels in the late

summer months may be linked to the higher water temperatures observed within the hatchery at this time, reaching > 10°C for the first time in the study. Temperature is well-known to have mediating effects on lipid metabolism (Greene and Selivonchick, 1987), although both diet and temperature appear to have been eliminated as a direct mediator of smoltification-associated lipid mobilisation (Sheridan *et al.*, 1985b).

In addition to a general increase in lipid mobilization during the parr-smolt transformation, there have been several reports of specific mobilisation of saturated or unsaturated FFA from lipid depots. Saddler *et al.* (1972) reported that the downstream migration of chum salmon (*O. keta*) was accompanied by a depletion of whole body monounsaturated fatty acids and an increase in proportion of polyunsaturated fatty acids, notably C22:6. Maintenance of these chum fry in freshwater led to a return to a freshwater type of lipid composition. Ota and Yamada (1974) noted that in the tissues of yearling Masu salmon, (*O. masou*), palmitoleic, oleic, linolenic and linoleic acids decreased over winter months whereas the polyunsaturated acids eicosatetraenoic (C20:4), eicosapentaenoic (C20:5), and docosahexaenoic (C22:6) increased. They further noted that seaward migrants in the spring had lower fatty acid levels, particularly palmitoleic and oleic, than non-migrant fish. As well, these migrants had lower tissue triglycerides and higher phospholipid and free sterols than the non-migrants. The authors suggested that in the seaward migrating fish, the monoenes may be preferentially consumed (for energy), and that, as smoltification nears completion, the consumption of polyenoic acids as well as saturated and monoenoic acids might increase. Sheridan *et al.* (1983, 1985a) also noted that tissue of freshwater parr of steelhead trout were characterised by low levels of polyunsaturated fatty acids and high amounts of linoleic (C18:2) acid whereas smolts possessed a more typically marine fatty acid

composition: high levels of polyunsaturated fatty acids with concomitant reduction of saturated fatty acid content. Again, the data suggested that fish undergoing smoltification may preferentially utilise saturated and monoenoic fatty acids early in smoltification, and only use the polyunsaturates near the time when outmigration is imminent. In addition to these studies on differential mobilisation of FFAs during smoltification, there are several reports on differential mobilisation of lipid depots. Thus, Tikhomirov (1978) noted that, during forced swimming of rainbow trout, liver lipids were mobilised much quicker than muscle lipid depots. Further, the age of the trout was also important, with yearlings mobilizing fats quicker than underyearlings and much quicker than three year-old trout. Plasma FFA levels in all three age groups increased within 1 hour of exercise. The author further noted that mobilisation of muscle lipid stores occurs only once carbohydrate stores are exhausted. Sheridan (1986) also noted that while T_4, GH and cortisol were all effective in lowering liver lipid concentrations in coho parr, none of the hormones were effective in lowering muscle lipid levels in parr or smolts. Sheridan *et al.* (1985b) further states that while mesenteric fat forms the primary lipid depot in juvenile coho salmon, the importance of this tissue decreases as smoltification proceeds. Jezierska *et al.* (1982) also noted that, during starvation in rainbow trout, the visercal mesenteric fat contributes a much larger amount to energy metabolism than either the muscle or liver depots. Further, he found that saturated fatty acids were preferentially mobilized from the mesentery, leading to an increase in the percantage of tissue mono- and poly-unsaturated fatty acids. In the liver, however, the monounsaturates were the first to be mobilized, while saturated and polyunsaturated fatty acids remained at a stable percentage. The muscle compartment exhibited yet a third pattern, with the percentage of monounsaturates decreasing, saturated fatty acids increasing, and polyunsaturates remaining stable.

In the 1982 data, there was a difference in saturated versus unsaturated FFAs, as seen by the ratio in the plasma. In the initial sampling weeks (February to mid-April), the ratio was quite low (between 2-3), whereas in late April to May there was a large increase in the levels of saturated FFA in the plasma. Again, the plasma level of these metabolites does not tell us what the kinetics are: ie., are saturated FFA levels increasing because of increased release from the depots or because of a large decrease in uptake by peripheral tissues. Sheridan *et al.* (1985a) also noted large increases in the saturated fatty acid levels in serum of smolting steelhead trout. The results of the above-mentioned studies, as well as the increased rate of condition factor decline, would suggest that increased mobilisation would be the primary cause, as suggested by the enzyme work of Sheridan *et al.* , (1985b). Increased mobilisation of saturated fatty acids, perhaps from the mesenteric fat depots, would lead to increases in tissue mono- and poly-unsaturated fatty acid levels and an increase in circulating saturated fatty acid concentration. Increased energetics at this time could be a reason why saturated FFAs are more prevalent. Numerous studies have shown increased metabolic rates during smoltification. Plasma levels of unsaturated FFAs did not display any such increases, although this does not mean that there were no changes in turnover rates of these compounds. The data in the literature, however, indicate that these compounds appear to be retained within the tissues, and are perhaps more important in the marine environment, as discussed in the next chapter.

The data from the 1983 study was different: no indication of differential mobilisation or release of saturated versus unsaturated fatty acids was observed in the plasma of the fish. The total saturated and unsaturated FFA plasma concentrations displayed very similar patterns over the 18 weeks of measurement. The ratio of plasma concentrations of saturated to unsaturated

FFA was fairly stable, although a slight increase could be seen over the initial 7-8 weeks, perhaps indicative of increased saturated FFA demand. It may be that the preferential release and utilisation of saturated fatty acids, if indeed this does occur, happened earlier in the parr-smolt transformation. As well, body lipids may already be low in these animals such that differential metabolism of the two classes fatty acids may be overridden. It was interesting, however, that in these 1983 fish plasma GH levels were correlated with saturated but not with unsaturated plasma FFA titers over the sampling period. The correlation between ratio of saturated/unsaturated FFA and GH levels in the plasma was even more striking. Conversely, in the pre-release fish of 1983, plasma levels of the unsaturated FFAs possessed the highest correlation with circulating GH levels, although no significance was observed. No correlations between plasma GH and FFA parameters were observed in the 1982 study, although the circulatory GH levels showed a much higher 'r' value to the saturated, total and ratio parameters. Whether GH in fish has a different effect on the mobilisation of saturated or unsaturated fatty acids is unknown. Clejan and Schulz (1986) have demonstrated that, in rats, GH enhances mitochondrial oxidation of polyunsaturated fatty acids. In the endothermic animals, polyunsaturated fatty acids, while important, are not as essential for the maintenance of membrane function as they are in the poikilothermic fish. It would be interesting if these respiration effects of GH are also observed in fish and whether they affect saturated or unsaturated FFA differently. Blake *et al*. (1984) demonstrated that Atlantic salmon smolts, as compared to parr, possessed highly increased levels of hepatic cytochrome c oxidase enzyme activity. This enzyme is a primary respiratory chain component. Additionally, these authors reported increased numbers of hepatic mitochondria. These results point to a highly increased capacity for aerobic respiration and, thus, increased capacity for fatty acid oxidation.

The results from my sampling studies confirm that growth hormone levels do increase during the parr-smolt transformation, particularly as smoltification nears completion. This result is similar to the increases in plasma cortisol and thyroid hormone levels as smoltification nears completion, perhaps indicating a complex interaction between these factors. The intricate and sometimes inconsistent interrelationships between the various plasma and tissue metabolites during smoltification made assigning a definite function to the enhanced growth hormone secretion a difficult task, although in both 1982 and 1983 the subsequent carbohydrate mobilisation appears to be important. The strong increase in saturated fatty acid levels seen in the 1982 study further indicate that a primary role for GH in these fish may be to enhance mobilisation of energy supplies. The continued elevation in plasma levels in the desmolting fish also seem to indicate a role for GH in this reverse transformation. Although the limited background information does not allow me to make any strong conclusions, there may be increases in metabolic rates in these fish as the growing season ends.

It appears then, that successful transformation from a freshwater parr to a seawater smolt is dependent to some degree upon GH. However, smoltification can only truly be termed successful if the fish are capable of rapid adaptation to the seawater environment. The second area of my research was on the possible actions of GH in this short-term response.

CHAPTER II

ROLES OF GROWTH HORMONE DURING SEAWATER ADAPTATION

INTRODUCTION

Smoltification may be considered a preparative phenomenon, a *pot pourri* of behaviourial, morphological and physiological changes serving to pre-adapt the young salmonid to a seawater existence (Hoar, 1976;Folmar and Dickhoff, 1980;Wedemeyer *et al.*, 1980). Downstream migration may, in fact, be stimulated to some degree by the stress the fish may be under by being somewhat adapted to a saltwater environment while still being in a freshwater habitat (Simpson, 1985;Langhorne and Simpson, 1986). Many studies have demonstrated peaks in plasma levels of hormones 'involved' in smoltification, enzyme activities, and in seawater preference or tolerance occur several weeks before downstream migration (Conte and Wagner, 1965;Conte *et al.*, 1966;Otto, 1971;Wagner, 1974). The young fish may, therefore, be well adapted to the hyperosmotic marine environment before the actual out-migration. However, transfer to seawater prior to completion of the smolt transformation will lead to high mortality rates or reduction or cessation of growth ("stunting"), or both, of a large proportion of the population (Mahnken, 1973;Bern, 1978;Fryer and Bern, 1979).

As pre-adapted as the migrant smolts are, however, actual entry into the marine environment constitutes a separate phenomenon, one which results in a considerable amount of stress on the smolt, particularly in the first 24-36 hours post-entry. In the wild, downstream migrants may spend a substantial period of time in the brackish waters of the river mouth, perhaps as a behaviourial mechanism to avoid or alleviate the stresses of entry into full-strength seawater (ie., to 'prime' the system). The estuaries may also act as a staging area, accelerating less

developed 'smolts' (perhaps seen in the 1983 study) to reach the degree of osmoregulation required before successful entry to the marine environment. In addition to the activities discussed in Chapter 1, lunar cycles in physiological and behaviourial (e.g., downstream migration) patterns (Mason, 1975;Grau *et al.*, 1982; Youngson *et al.*, 1983;Sweeting *et al.*, 1985) may be proximal timing mechanisms to reach the rivermouth when tides will provide high amounts of brackish water, as well as increasing protection from predation by other fish or birds. Lunar cycles in out-migration may also be tied in with food cycles in the marine environment. Entry into hyperosmotic environments results in rapid changes is several physiological parameters, including elevations in plasma electrolytes (e.g., Na^+ and Cl^-), dehydration of tissues, etc., which are largely passive. The ability to rapidly recompensate for these changes is indicative of the preparatory aspects of the parr-smolt transformation. While the mechanisms of adaptation are present, it may require a certain amount of time to 'activate' the various systems to ensure survival and growth in this new environment. Clarke and Blackburn (1977) and Blackburn and Clarke (1987) have suggested that survival and return of plasma Na^+ concentrations to freshwater levels within 24-36 hours of seawater entry is an accurate test of completed smoltification. This ability to regulate plasma osmolality in a hyperosmotic environment is due, to a significant amount, to increases in the level of the Na^+,K^+-ATPase enzyme. This enzyme, involved in electrolyte regulation, is found in many cells, including red blood cells, renal tissues, and intestinal tissues. The majority of the plasma Na^+ regulatory capacity is, however, contained within the gill tissues, specifically within the chloride cells. Na^+,K^+-ATPase, along with other (osmo)regulatory enzymes (e.g.,carbonic anhydrase, Ca^{++}-ATPase, Mg^{++}-ATPase), are also found in the respiratory epithelial cells of the gill tissue. Chloride cells, but not the respiratory epithelial cells, undergo hyperplasia as

well as differentiation during both smoltification and seawater adaptation (Wedemeyer *et al.*, 1980). This complex interaction of the chloride cells and the external environment has stimulated a large amount of research, of which suprisingly little is concerned with the endogenous control mechanisms utilized by the adapting fish, ie., the endocrine system.

The involvement of the pituitary in the development of iono(osmo)regulatory mechanisms in salmon was shown by Hoar (1951), who noted that injections of mammalian anterior pituitary extract stimulated chloride cell activity in Atlantic salmon parr (c.f. Wedemeyer *et al.*, 1980). By 1956, D. C. W. Smith had shown that mammalian growth hormone injections enhanced the seawater survival in brown trout. This enhanced survival following seawater transfer has also been shown for Atlantic salmon (Komourdjian *et al.*, 1976), sockeye salmon (Clarke *et al.*, 1977) and amago salmon (Miwa and Inui, 1985). Nagahama *et al.* (1977) and Nishioka *et al.* (1982) reported that the somatotroph cells within the pituitaries of seawater-adapted salmon smolts demonstrated characteristics suggestive of increased secretion as well as increased synthetic activity. Somatotroph cells were also markedly degranulated in stickleback, *Pungitius pungitius*, transferred to seawater (Benjamin, 1978). An osmo(iono)regulatory function for GH in hypersaline environments is an interesting counterpart to the well-known osmoregulatory role of GHs' "sister" hormone, prolactin, in in freshwater environments (eg., Schreibman and Kallman, 1966). However, due to the length the GH treatments, most of the studies could not discriminate whether the effect of GH was a direct action on some adaptive system (ie., osmoregulation) or whether the enhancement was an indirect action derived from a general increase in body size, as size has also been shown to be directly related to seawater survival (Farmer *et al.*, 1978). Further, GH may act in an indirect fashion via its general stimulation of hyperplasia (Cheek, 1971).

Clarke *et al.* (1977) and Collie *et al.* (1985), working with sockeye salmon and rainbow trout respectively, demonstrated that GH treatment led to significantly lower plasma Na^+ levels (compared to controls) 24 hours post-exposure, suggesting that GH treatment may act on the osmoregulatory system in a direct fashion. However, both of these studies (and others as well) were initially concerned with the ability of GH to enhance the growth and/or smolt index of the fish, and as such, consisted of long-term injection of GH. The authors were thus unable to eliminate the difference in size between the controls and the GH-injected fish, ie., an indirect effect. Additionally, most of these studies were done utilizing mammalian growth hormone, which introduced the question whether these results would also be seen using homologous growth hormone or whether the enhanced survival in seawater resulted from either impurities in the injections or from non-specific hormone-receptor interactions. For example, Clarke *et al.* (1977) demonstrated that, at 1.0 μg/gm, ovine PRL had somatotrophic activity in juvenile sockeye (albeit, less potent than *Tilapia* growth hormone), whereas *Tilapia* PRL was ineffective. The authors also reported that *Tilapia* GH, bovine GH, and ovine PRL all reduced plasma sodium levels compared to controls 24 hours post-transfer of juvenile sockeye salmon to seawater, whereas *Tilapia* PRL was again without effect. Thus, the question of growth hormone's involvement in osmoregulation was still very much of a speculative nature. With the purification and subsequent availability of teleost growth hormones in the late 1970's, it became possible to examine some actions of homologous or near-homologous GHs, not only in terms of growth-enhancing ability, but also of specific metabolic activities. Fryer (1979) demonstrated the presence of specific receptors for both *Tilapia* and coho growth hormone in the gill microsomal fraction of coho salmon smolts, suggesting that this hormone may indeed have some direct osmoregulatory capacity. Additionally, Fryer and Bern (1979) demonstrated that the gill

capacity for binding GH was reduced in the so-called "stunts", although the fish eventually were able to regulate plasma Na^+ levels effectively. Thus, evidence continued to indicate that GH, addition to its growth-promoting abilities, may have a direct effect on the osmoregulatory mechanisms within the gill tissue.

If growth hormone is involved in seawater adaptation, then it seemed likely that , I conducted te experiments. The initial experiment was to examine the effects of short-term (24 h) seawater exposure on the plasma profile of GH, . Previous studies on plasma GH levels associated with seawater adaptation of salmon, utilizing heterologous assays, had led to contradictory results. The purpose was to examine whether hypersaline environments induced hyper-secretion of GH into the plasma, as suggested by histological studies. If GH was important in osmoregulatory function, one might expect changes in plasma concentration of GH over the time course of adaptation. A sampling period of 24 hours was chosen for this initial study, in view of th 24-hr Na^+ regulatory control proposal outlined by Clarke and Blackburn (1977). As well, I also assayed for possible changes in both plasma glucose and amino acid nitrogen, two classes of organic compounds with which GH has been assigned a regulatory role. Plasma glucose levels in fish have been shown in many studies to be strongly increased by increased plasma cortisol. Additionally, plasma cortisol has been demonstrated to increase rapidly upon seawater entry in salmonids. Further, the measurement of metabolic parameters associated with GH provides a mechanism to alleviate some of the drawbacks of examining only the plasma level of a hormone. The level of a hormone in the blood does not always reflect the true physiological/metabolic situation, which will be a result of many factors, including synthesis, secretion, receptor uptake, degradation, carrier-bound, etc. (Murat, *et al.*, 1981).

The second experiment, compliant with the results from the first experiment, was conducted for a longer period of time (72 hours). This experiment was done to further examine the changes seen in plasma GH levels upon seawater exposure and to examine possible roles of GH in osmoregulation in seawater. Thus, in this experiment, plasma Na^+ levels were assayed, along with plasma glucose. In addition to the plasma glucose, samples of liver and muscle tissue were analysed for glycogen and glucose levels. Further, plasma levels of individual free fatty acids were assayed. In fish, as in terrestial vertebrates, lipids serve as an important energy source. GH induced lipolysis ahs been demonstrated in a number of vertebrates. Further, both from an energetic and precursor point of view, marine fish demonstrate significant differences in FFA from their FW counterparts, and some of these differences may manifest themselves during SW adaptation. As a sub-experiment within this second seawater exposure, a group of fish were injected with somatostatin (SRIF), the biological inhibitor of the release of GH from the pituitary somatotrophs (Brazeau *et al.*, 1973). This hormone has been found in all vertebrate classes, including fish (see Plisetskaya *et al.*, 1986). By injecting SRIF into these fish just prior to seawater exposure, I hoped to decrease the availability of GH for any putative iono(osmo)regulatory actions. By assaying for the same parameters as in the non-injected fish, the consequences of this inhibition of GH release to the normal adaptation to seawater might be observed.

METHODS

Seawater Transfer Experiment No. 1, May 1983

Juvenile coho salmon, obtained from Capilano Hatchery in North Vancouver, were maintained under natural photoperiod and temperature (7°C) in a 120L flow-through tank until the experiment commenced (approx. 30 days). Feeding was twice daily, *ad libitum*, using M & H Trout Feed (Surrey,B.C.). On May 5, the fish were divided into 2 groups (n=32) of equal weights and lengths (12.11 ± 0.348 gm; 11.14 ± 0.098 cm) and placed in identical 120L flow-through tanks for 7 days. Feeding was stopped 2 days prior to seawater exposure to eliminate any feeding differences between groups as a result of a change in salinity. At hour 0, eight fish were sampled from each tank, as previously described. Seawater (29 ppt; 8°C) was then allowed to flow into one of the tanks. Complete replacement took approximately 2.5 hours. Fish were then sampled from each tank at 6, 12 and 24 hours after time 0. The blood was centrifuged, hematocrits read, and the plasma stored at -20°C until assayed for GH, amino acid nitrogen, glucose and free fatty acids.

Seawater Transfer Experiment No. 2, June 1985

Juvenile coho salmon were obtained from Capilano Hatchery in North Vancouver, transported to the lab, and maintained under natural photoperiod from early April to June, 1985. Water temperature in March was 5.0°C and rose to 11.0°C by June. Ten days prior to the experiment (June 20), 130 smolts (as determined by silvery coloration and absence of parr marks) were removed and divided into two groups of equal weights and lengths (11.55 ± 2.06 gm; 11.54 ± 0.60 cm) Both groups were placed in 120L flow-through tanks supplied with freshwater (0.8L/min). Fish in the "seawater" tank were further subdivided into three groups: group 1, the seawater controls (n=50); group 2, the saline-injection group (n=15); and group 3, the

somatostatin-injection group (n=15). Group 3 fish were separated from groups 1 and 2 by a clear, perforated sheet of plexiglass. Both groups 2 and 3 were further identified by removal of the adipose fin at time of weighing. Fish in both the "seawater" and "freshwater" (n=50) tanks were fed twice daily, *ad libitum*, with M & H Trout and Salmon Feed No. 5 Crumble (Surrey,B.C.) until 2 days prior to the experiment.

At hour 0, prior to seawater exposure, seven fish were sampled from the freshwater control and the seawater control groups. Blood was collected in heparinized capillary tubes as described previously. Following centrifugation, hematocrits were read and the plasma fraction immediately frozen on dry ice. The fish were killed by severing the spinal cord immediately caudal to the head region. The peritoneal cavity was exposed and the fish were placed on dry ice. The body and plasma samples were subsequently stored at -20°C until assayed.

Immediately following this sampling at hour 0, fish from groups 2 and 3 were injected intraperitoneally with either 0.50 mL ice-cold Cortlands teleost saline (0.1M; 7.25 g/L NaCl, 0.38 g/L KCl, 0.162 g/L $CaCl_2$, 0.23 g/L $MgSO_4:7H_2O$, 1.0 g/L $NaHCO_3$, 0.41 g/L $NaH_2PO_4:H_2O$, 1.0 g/L glucose; pH 8.1 with 2.0M NaOH) or somatostatin (SRIF; Sigma) (2.0 µg/10 g body weight) in 0.50 mL ice-cold Cortlands. Seawater (33 ppt; 11°C) was then allowed to flow into the tank containing groups 1, 2, and 3 such that complete turnover required approximately 2 hours. Both tanks were subsequently converted from a flow-through system to a recirculating system for the duration of the experiment. Seven fish from each of the four groups were subsequently sampled at 6 and 12 hours, then, from the freshwater and uninjected seawater groups at 24, 36, 48, and 72 hours post-exposure. Plasma and tissue samples were obtained at each sampling period as above.

Assay techniques

Plasma sodium levels (for experiment 2) were determined by flame emission spectrophotometry using a Pye Unicam SP131 Atomic Absorption Spectrophotometer. Five μL of plasma were diluted in 10.0 mL of distilled water. Duplicates of each sample were analyzed against a standard curve.

Tissue glycogen and glucose and plasma glucose, amino acid nitrogen, growth hormone, and free fatty acids were determined as outlined in Chapter 1.

Statistics

Results from the seawater exposure experiments were analyzed for within group differences by oneway analyses of variance (ANOVA), using Student Newman-Keuls multiple range tests. Significance was assumed at the 0.05 level. Differences between groups were analyzed by Student's T-test, with significance assumed at the 0.05 level.

Correlations were done utilizing Pearsons' correlations, with significance assumed at the 0.01 level.

RESULTS

1. SHORT TERM EXPOSURE

Plasma hematocrits (Figure 14a) were not significantly different between freshwater and seawater exposed groups at 0, 6, or 12 hours post-exposure. Seawater exposed fish had lower hematocrits than freshwater controls at hour 24, possibly due to influx of water from the cellular compartment. There were no hematocrit differences within either the freshwater control or seawater exposure groups at any time. Also, the 24 hour seawater sample consisted of only 4 fish, due to one mortality and 3 missing fish. Seawater exposure resulted in a dramatic rise in plasma GH levels (Figure 14b). The increase in plasma GH within the seawater group was not significant after 6 hrs, but the levels were higher than the 0 and 6 hr sampling at both 12 and 24 hours. There were no significant differences between control and experimental fish at 0 or 6 hours, however, seawater-exposed fish did have higher plasma GH levels than freshwater controls at 12 and 24 hours post-exposure. There were no significant differences in plasma GH at any time within the freshwater control group.

Plasma glucose levels are shown in Figure 14c. There were no significant differences within or between the two groups at 0 or 6 hours post-exposure. However, at 12 post-exposure, the freshwater plasma glucose levels were lower than those values seen in the seawater group, whereas at 24 hours the plasma glucose in the freshwater sample was higher than the levels seen in the seawater fish. In addition, this freshwater 24 hour level was higher than the freshwater 12 hour level. At no time were there any significant differences in plasma glucose within the seawater exposed fish.

The plasma free amino acid nitrogen (pAAN) concentration profile is shown in Figure 14d. Plasma AAN titers were not

Figure 14: Plasma parameters in juvenile coho salmon exposed t seawater for 24 hours. All points represent means (n=6-8) ± SI Closed circles represent freshwater controls and open squares represent fish exposed to seawater. Stars (*) indicate valı significantly ($P < .05$) different than controls. Within grou differences are indicated by letters, such that means with t same letters are not significantly different.

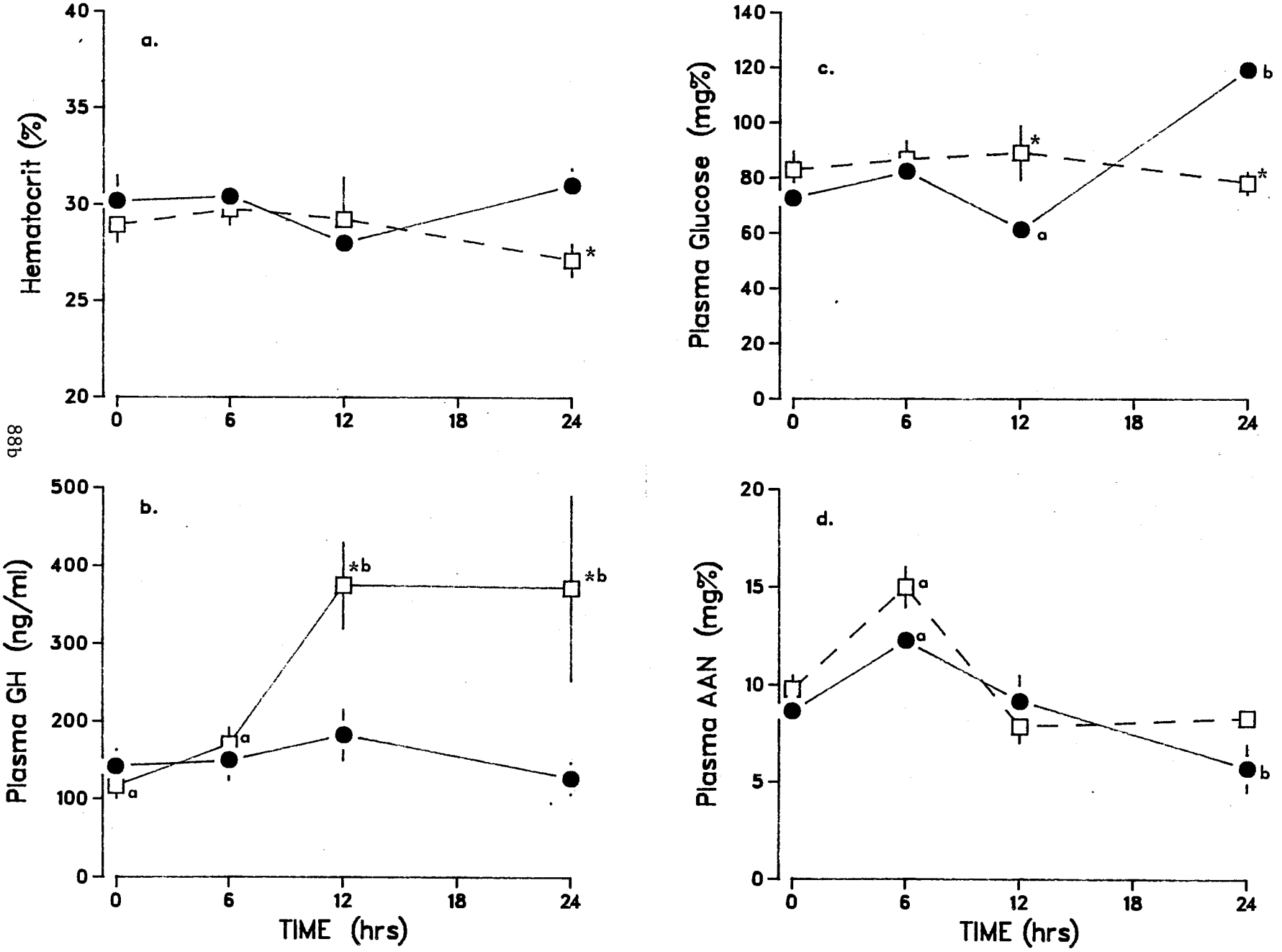

a.
Hematocrit (%)
40
35
30
25
20
0
6
12
18
24
*
b.
Plasma GH (ng/ml)
500
400
300
200
100
0
*b
*b
a
a
TIME (hrs)
c.
Plasma Glucose (mg%)
140
120
100
80
60
40
20
0
b
a
*
*
d.
Plasma AAN (mg%)
20
15
10
5
0
a
a
b
TIME (hrs)

Table 5: Correlations between parameters of the 24 hour seawater adaptation. The abbreviations are as described in the text. The correlations are one-tailed where single stars (*) represent ($P \leq .01$) and double stars (**) indicate ($P \leq .001$). See text for detailed discussion.

A. Freshwater

	Weight	Length	Cond. Factor	Hemato	Plasma GH	Plasma AAN	Plasma Glucose	Plasma Sodium
Weight	1.0000	.9104**	-.2112	.6077**	-.4392*	.3485	.2848	.3069
Length		1.0000	-.5892**	.5368**	-.3577	.2313	.3051	.2390
Cond. Fact.			1.0000	-.0820	-.0876	.1668	-.1903	.2644
Hemato				1.0000	-.3973	.1445	.4900*	.1822
Plasma GH					1.0000	-.2056	-.1264	-.2871
Plasma AAN						1.0000	-.4348	-.0946
P-Glucose							1.0000	.1902
P-Sodium								1.0000

B. Seawater

	Weight	Length	Cond. Factor	Hemato	Plasma GH	Plasma AAN	Plasma Glucose	Plasma Sodium
Weight	1.0000	.8325**	.4262	.5779**	-.2195	-.0234	-.0173	.1218
Length		1.0000	-.1369	.2534	-.0512	.1356	-.3347	.1185
Cond. Fact.			1.0000	.5767**	-.3629	-.2388	.5427**	.0862
Hemato				1.0000	-.2790	-.0784	.1985	-.2774
Plasma GH					1.0000	-.3320	-.1371	-.6043
Plasma AAN						1.0000	-.4549	-.0617
P-Glucose							1.0000	.1525
P-Sodium								1.0000

different between the freshwater and seawater groups at any time in the experiment. Both groups exhibited a peak in pAAN at hour 6. The seawater peak was higher than all other times, whereas the freshwater peak was higher only than the 24 hour level.

Both weight and length in the freshwater fish were positively correlated with hematocrit (Table 5) over the 24 hour sampling period, whereas in the seawater fish only weight demonstrated a correlation with hematocrit. Weight and length in freshwater fish were negatively correlated with plasma GH levels, as did hematocrit. Plasma glucose, however, was positively correlated with GH in FW. In the seawater-exposed fish, there were no correlations between weight-GH, length-GH or hematocrit-GH. However, there were positive correlations between condition factor and hematocrit and between condition factor and plasma glucose.

2. LONG-TERM (72 hour) EXPOSURE

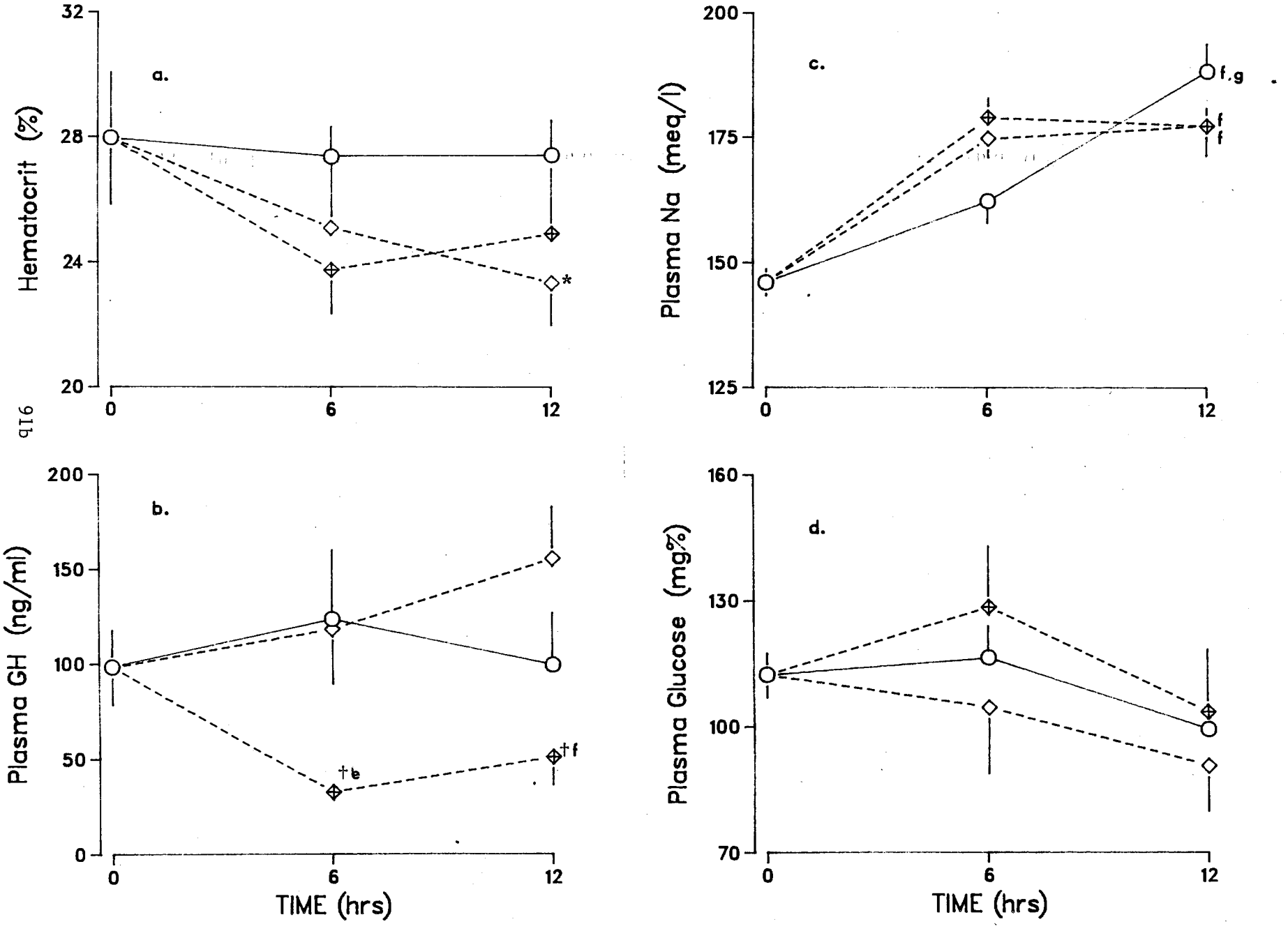
In this study, the injections of saline and somatostatin (SRIF) comprise a separate sub-experiment within the 72-hour exposure experiment and will therefore be considered separately.

A. Effects of Somatostatin on 12-hour SW exposure

Hematocrits (Figure 15a) in the seawater control group (those fish which received no injection) showed no change over the first 12 hours of the exposure, as in the 24-hour exposure experiment. Injection of saline, however, led to a significantly lower hematocrit than controls at 12 hours. This level was not different than the 0 hour level. The group injected with SRIF had no differences from the saline group at any time nor were the levels at 6 and 12 hours different from the 0 hour level.

Plasma growth hormone levels in the control group did not show any differences over the initial 12 hours of seawater

Figure 15:Plasma parameters in SRIF-treated juvenile coho salmon exposed to seawater for 12 hours. Open circles represent uninjected controls. Open diamonds represent fish injected with 0.5 ml saline prior to seawater exposure and hatched diamonds represent fish injected with somatostatin in 0.5 ml saline prior to exposure. Significant differences between saline-injected group values and non-injected controls are signified by (*); differences between the saline-injected group and the somatostatin-injected group are represented by (†). Within group differences are signified by letters: 'e' represents differences between 0 and 6 hours post-exposure, 'f' designates differences between 0 and 12 hours post-exposure while 'g' signifies differences between 6 and 12 hours post-exposure. All significant differences ($P < .05$).


a.
Hematocrit (%)
32
28
24
20
0
6
12
*
b.
Plasma GH (ng/ml)
200
150
100
50
0
†e
†f
TIME (hrs)
c.
Plasma Na (meq/l)
200
175
150
125
f,g
f
f
d.
Plasma Glucose (mg%)
160
130
100
70
TIME (hrs)

91b

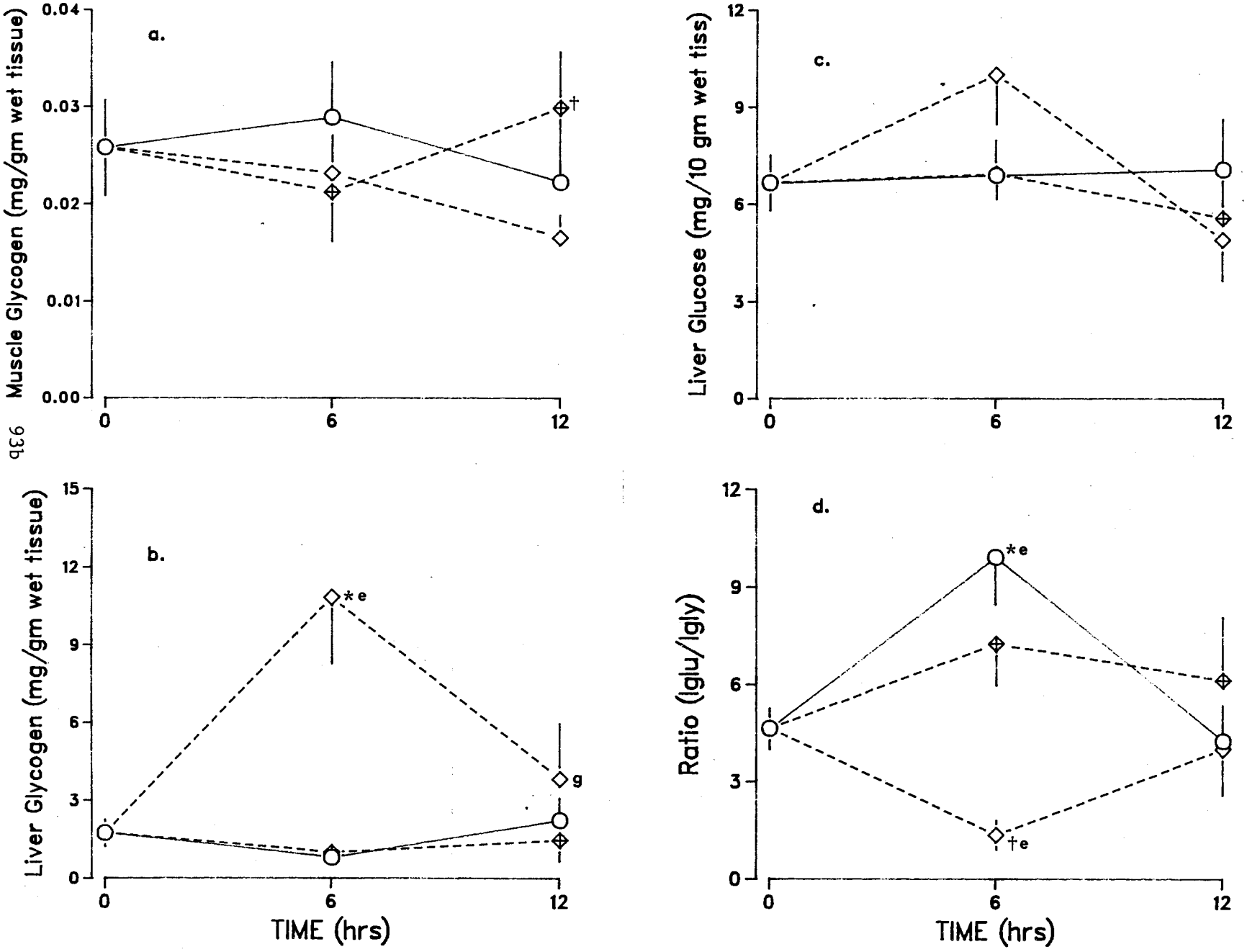
exposure (Figure 15b). The saline-injected group was not different from the control group at any time, nor were there any differences over time in this group. In contrast, the SRIF-injected group had lower levels of plasma GH than saline-injected fish at both 6 and 12 hours. These levels were also lower than the pre-exposure or 0 hour level.

The plasma sodium levels in all three groups (Figure 15c) exhibited a rapid rise in concentration by 6 hours of seawater exposure. At 12 hours post-exposure, all three groups continued to exhibit higher levels than pre-exposure, but only the control group at 12 hours had higher levels than at 6 hours. Neither the saline nor the SRIF injections had any significant effect on plasma sodium levels at 6 or 12 hrs. (Note: At 6 hours, the SRIF-injected group had plasma sodium levels higher than the uninjected control group).

There were no differences in plasma glucose levels within (ie., over time) or between groups at any time in the first 12 hours of seawater exposure (Figure 15d).

Levels of muscle glycogen did not show any differences within any of the groups over the 12 hours of seawater exposure (Figure 16a). There were no differences between any of the groups at 6 hours. At 12 hours post-exposure, the SRIF-injected group had higher levels than the saline-injected group. In the liver, neither the control or the SRIF-injected group had any differences in glycogen levels over time (Figure 16b). The saline-injected group however exhibited a large increase in glycogen concentration at 6 hours post-exposure. This level was higher than both control and SRIF-injected values at 6 hours, and was also significantly higher than levels in the saline-injected group at 0 and 12 hours. Although the free glucose levels in livers of the saline-injected group also exhibited a rise at 6 hours post-exposure (Figure 16c), this increase was not

Figure 16:Changes in tissue carbohydrates in SRIF-treated juvenile coho salmon exposed to seawater for 12 hours. Symbols and letters as assigned in Figure 14. All significant differences ($P < .05$).


a.
Muscle Glycogen (mg/gm wet tissue)
0.04
0.03
0.02
0.01
0.00
†
0
6
12
c.
Liver Glucose (mg/10 gm wet tiss)
12
9
6
3
0
0
6
12
b.
Liver Glycogen (mg/gm wet tissue)
15
12
9
6
3
0
*e
g
0
6
12
TIME (hrs)
d.
Ratio (Iglu/Igly)
12
9
6
3
0
*e
†e
0
6
12
TIME (hrs)

significant. There were no differences in this parameter within or between groups at any time. The ratio of glucose to glycogen in the liver did show significant differences within and between groups (Figure 16d). The saline-injected group at 6 hours possessed a lower ratio than either the control or SRIF-injected groups, probably the result of the large increase in liver glycogen. This ratio was also lower than the ratio at 0 hours. There were no differences between the groups at 12 hours post-exposure. In the control group, the ratio at 6 hours was significantly different than the pre-exposure ratio but not the ratio at 12 hours.

The plasma levels of individual saturated free fatty acids are shown in Figure 17.[1] Levels of lauric acid (C12:0)(Figure 17) in both injected groups were dramatically lower than pre-exposure and control group levels at 6 and 12 hours post-exposure. The control group did not demonstrate any differences over the 12 hours. Tridecanoic acid (C13:0) was also lower than pre-exposure levels in both injected groups at 6 and 12 hours post-exposure. Additionally, at 6 hours the SRIF-injected group had a lower level of tridecanoic acid than the saline-injected group. At 12 hours,the saline-injected group had a lower level of tridecanoic acid than the control group and the SRIF-injected group level was higher than the level at 6 hours. Myristic acid (C14:0) also demonstrated a rapid decline in plasma levels by 6 hours but only in the SRIF-injected group, whose values were also lower than pre-exposure levels at 12 hours. The saline-injected group had lower values than 0 hour at 12 hours post-exposure. This level at 12 hours was also lower than the control levels at this time. There were no differences within the control group over time. There were also no differences between the two injection groups at any time in this parameter. The plasma levels of pentadecanoic acid (C15:0) showed little difference over time in any group. The

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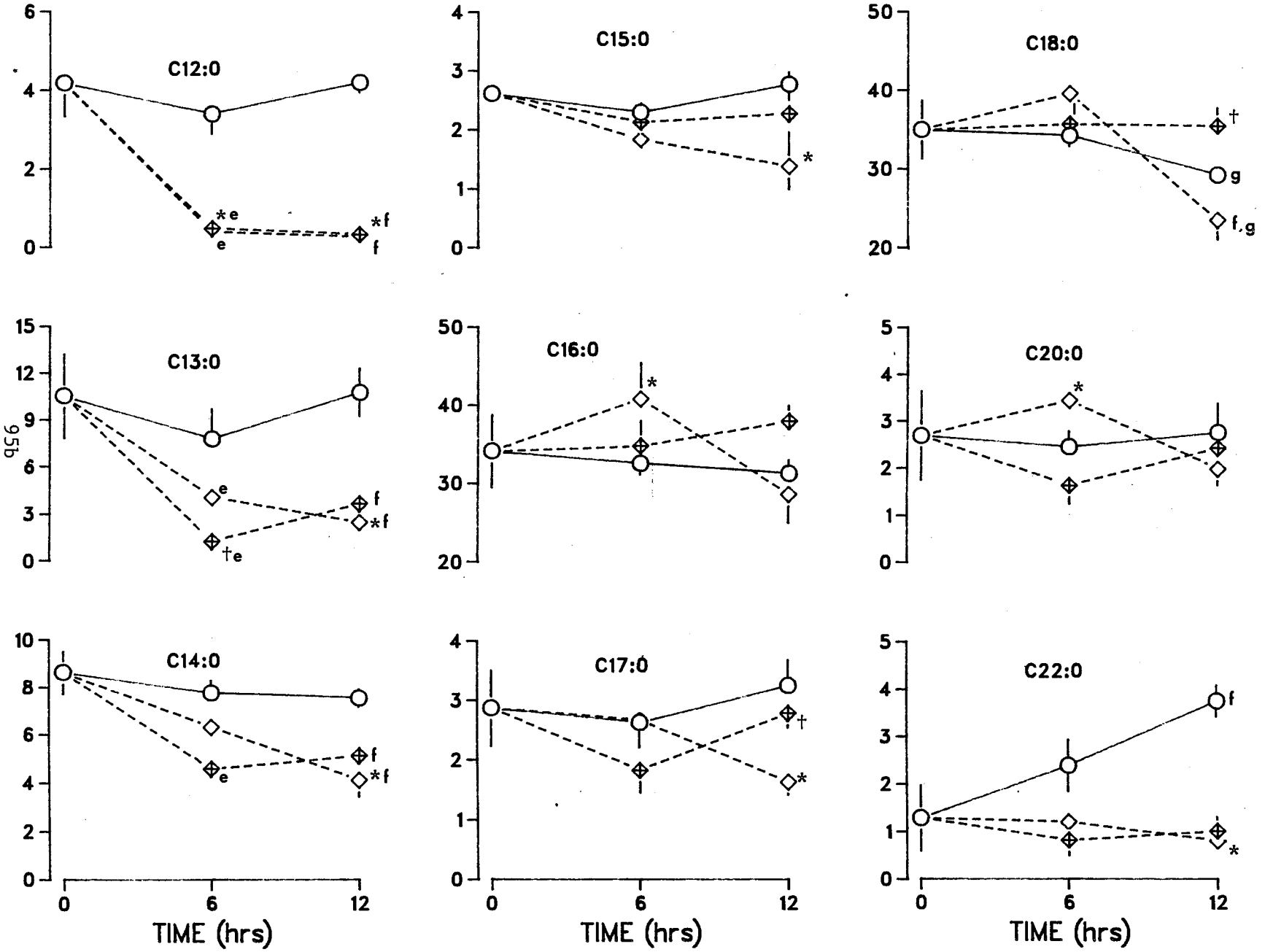
[1]See Table 1 for detailed description of fatty acid nomenclature.

Figure 17:Plasma saturated free fatty acids in SRIF-treated juvenile coho salmon exposed to seawater for 12 hours. Symbols and letters are as assigned in Figure 14. All plasma values are in in ng FFA/μL plasma. All significant differences ($P < .05$).

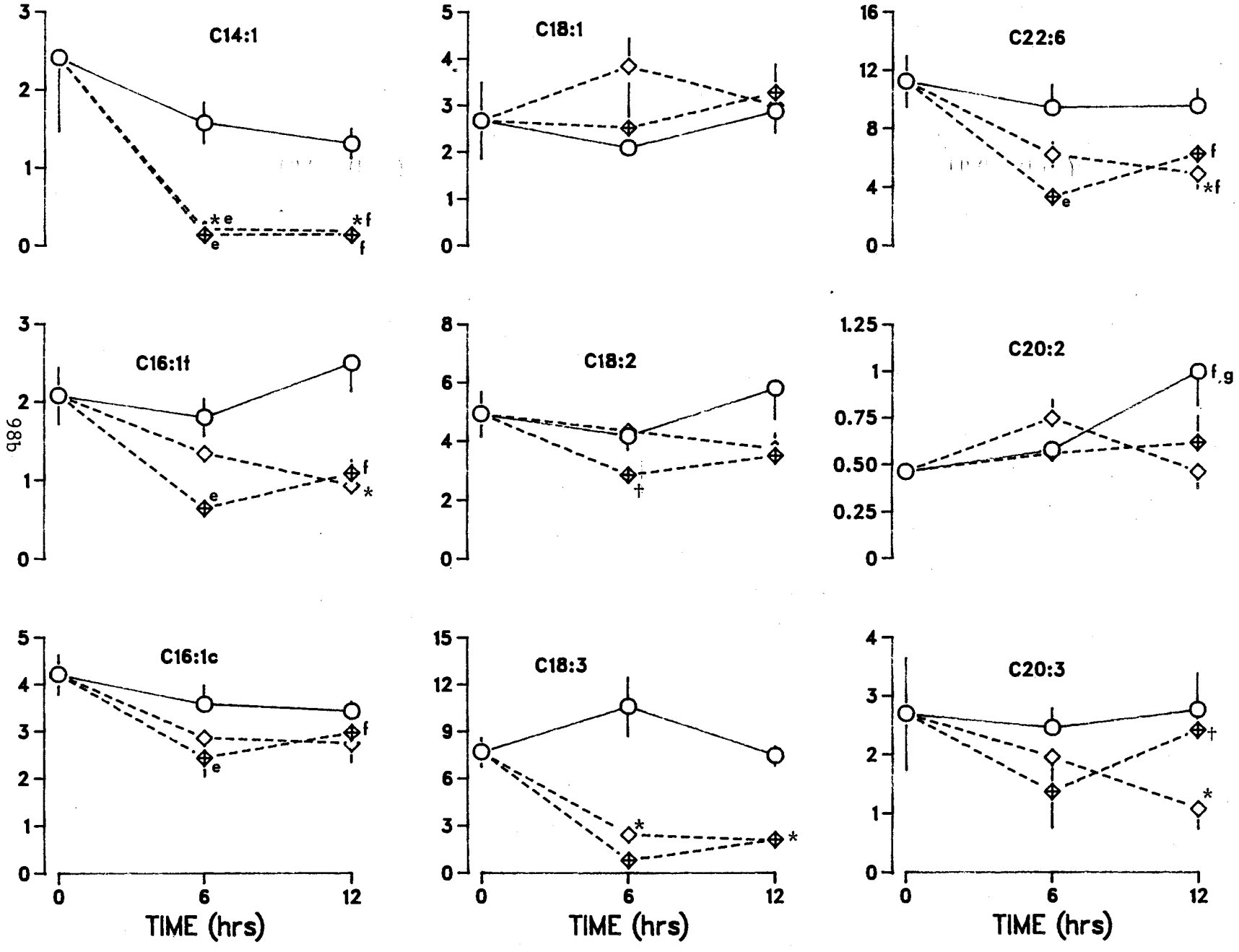

C12:0
C15:0
C18:0
C13:0
C16:0
C20:0
C14:0
C17:0
C22:0
TIME (hrs)
TIME (hrs)
TIME (hrs)

only difference was between the control and saline-injected groups at 12 hours post-exposure. This low level in the saline-injected group was not different than pre-exposure levels. Palmitic acid (C16:0), along with stearic acid (C18:0), exhibited the highest levels of nonesterified fatty acid, and appeared to be fairly constant within the control group The saline-injected group had a peak at 6 hours post-injection which was higher than the control group. There were no other differences in this parameter in the 12 hours. Heptadecanoic acid (C17:0) also demonstrated little variation over time in any of the three groups. At 12 hours the saline-injected group had plasma levels lower than the control group, whereas the SRIF-injected group was significantly higher than the saline-injected group. Stearic acid, along with palmitic, heptadecanoic and arachidic (C20:0) acids, demonstrated a different pattern than seen in the lower carbon fatty acids. These high carbon fatty acids, in addition to being at higher levels, did not exhibit the decline in plasma levels seen at 6 hours in the injection groups. There were no differences in stearic acid level at 6 hours within or between groups. At 12 hours however, the saline-injected group value was lower than the levels seen at 0 and 6 hours. The SRIF-injected fish had higher plasma concentrations than the saline-injected group at 12 hours. The level after 12 hrs in the control group was lower than the level seen at 6 hours in this group. Arachidic acid demonstrated little variation within or between groups at any time, with the only difference seen at 6 hours between the control and saline injected groups. Behenic acid (C22:0) demonstrated a somewhat unique plasma profile, in that levels of this fatty acid increased in the uninjected control group over the 12 hrs of exposure. The level at 12 hrs. in this group was higher than either the pre-exposure titer or the level in the saline-injected group at this time. Neither of the injected groups displayed much difference over the 12 hrs of sampling and there were no differences between them.

Plasma levels of the unsaturated free fatty acids are shown in Figure 18. Myristoleic acid (C14:1) had a dramatic decline in both injection groups at 6 hours post-exposure. These low levels were also seen at 12 hours. Control levels, while showing a declining trend, showed no differences over time. Palmitelaidic acid (C16:1*trans*) had a similar decline in plasma levels over time, but only the 12 hour saline-injected level was lower than the control values. In the SRIF-injected group, both the 6 and 12 hour levels were lower than the pre-exposure level, but there were no differences from the saline-injected group. Palmitoleic acid (C16:1*cis*) plasma levels in the SRIF-injected group at 6 and 12 hours were lower than the pre-injection level. There were no differences within either of the other two groups, nor were there any differences between groups at any time. Oleic acid (C18:1) demonstrated no significant differences within or between groups at any time in the 12 hours of seawater exposure.

The plasma levels of the polyunsaturated free fatty acids are also shown in Figure 18. Linoleic acid (C18:2) did not show the decline in plasma level in the saline-injected group. The SRIF-injected group level at 6 hours was lower than the saline-injected group value at this time. There were no within group differences in this fatty acid. Linolenic acid (C18:3) exhibited a dramatic decline in plasma concentration in the two injection groups at 6 hours post-injection/exposure. The saline-injected group levels were lower than control levels at both 6 and 12 hours. The SRIF-injected group levels were lower than the saline-injected group level at 6 hours but not at 12 hours. However, the 12 hour level in this group was different than the 6 hour level. There were no differences within the control group at any time. Docosahexaenoic acid (cervonic acid) (C22:6) showed a pattern intermediate to the previous polyunsaturates. The saline-injected group did exhibit a decline but only the 12 hour value was different than the control group.

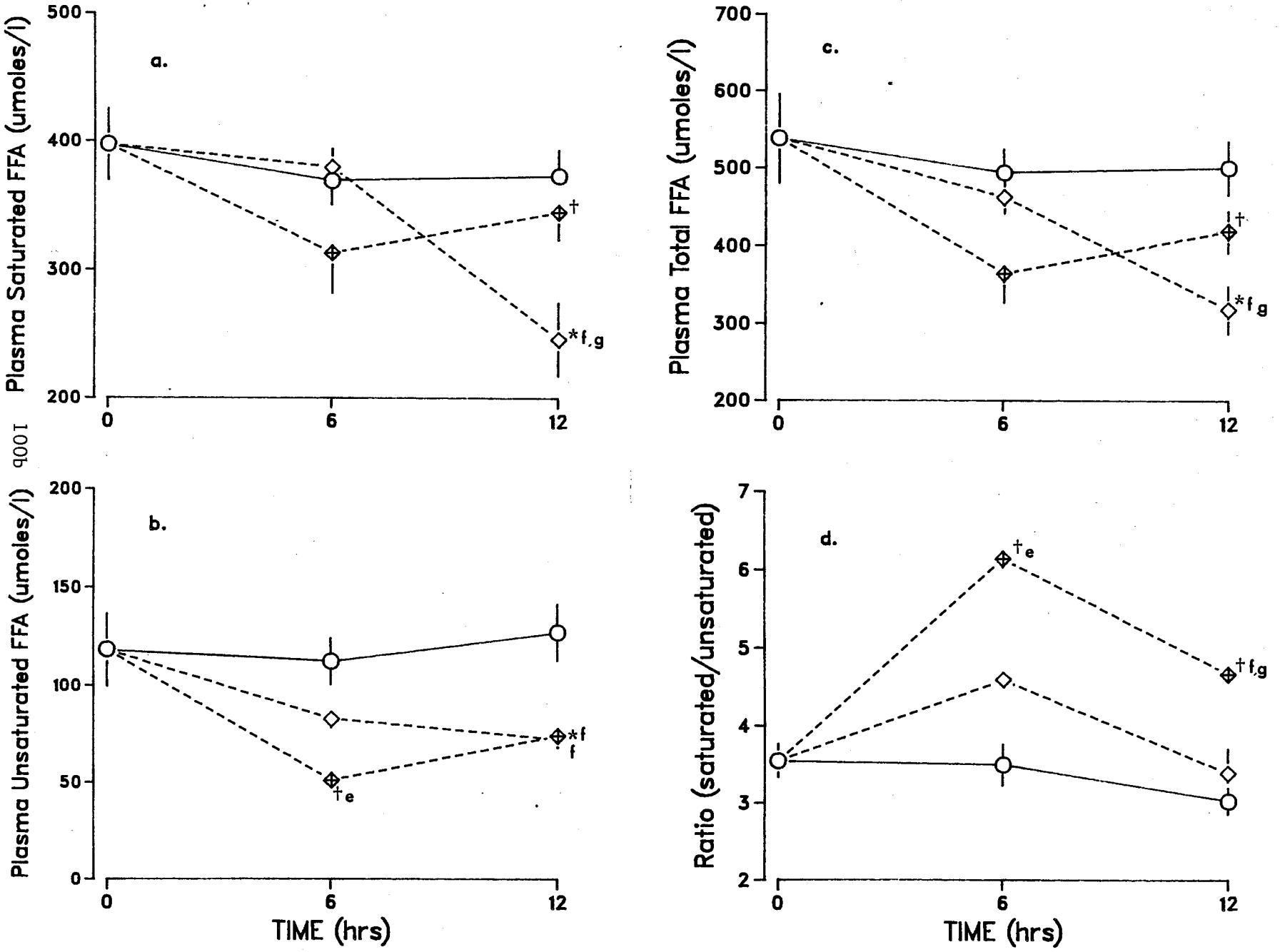
Figure 18:Plasma unsaturated free fatty acids in SRIF-treated juvenile coho salmon exposed to seawater for 12 hours. Symbols and letters are as assigned in Figure 14. All plasma values are in ng FFA/μL plasma. All significant differences ($P < .05$).


C14:1
C18:1
C22:6
C16:1f
C18:2
C20:2
C16:1c
C18:3
C20:3
TIME (hrs)
TIME (hrs)
TIME (hrs)

This 12 hour level was also lower than the 0 hour level. The SRIF-injected group had plasma levels lower than pre-injection values at both 6 and 12 hours, but did not exhibit any differences from the saline-injected group. Eicosadienoic acid (C20:2) was similar to its parent compound, C18:2, in that the SW control group exhibited a rise over the 12 hours of exposure, with the 12 hour value being higher than both the 0-hour and 6-hour level in this group. There were, however, no differences between the control and saline-injected groups nor the saline- and SRIF-injected groups at any time. There were also no differences within either the saline- or SRIF-injected groups over the 12 hours of exposure. C20:3, or eicosatrienoic acid, demonstrated no changes over time in the seawater uninjected group. The saline-injected group showed a steady decline over the sampling period, with the 12-hour value lower than both the control and the SRIF-injected groups at this time. There were no differences within groups at any time.

The total plasma FFA concentrations are shown in Figure 19a-d. (Note: the total concentrations referred to in this and the subsequent sections refer to totals of the individual fatty acids which I measured and not to a separate, gross assay or measurement). The saturated FFA in the control group did not show any differences over the 12 hours of this sub-experiment. The concentration of saturated free fatty acids in the saline-injected group were not different at 6 hours from control or pre-exposure levels, but the levels at 12 hours post-exposure in this group were lower than those seen at 12 hours in the control group as well as levels at 0 and 6 hours within the saline-injected group. The SRIF-injected group at 12 hours had higher levels than the saline-injected group. The total unsaturated fatty acid concentration, shown in Figure 19b, showed no differences within the control group over the 12 hours. Both the injection groups, however, showed dramatic declines by 6

Figure 19:Plasma free fatty acids in SRIF-treated juvenile coho salmon exposed to seawater for 12 hours. Symbols and letters are as assigned in Figure 14. Values for 19a, b, and c are expressed as μmoles FFA/L plasma. All significant differences ($P < .05$).

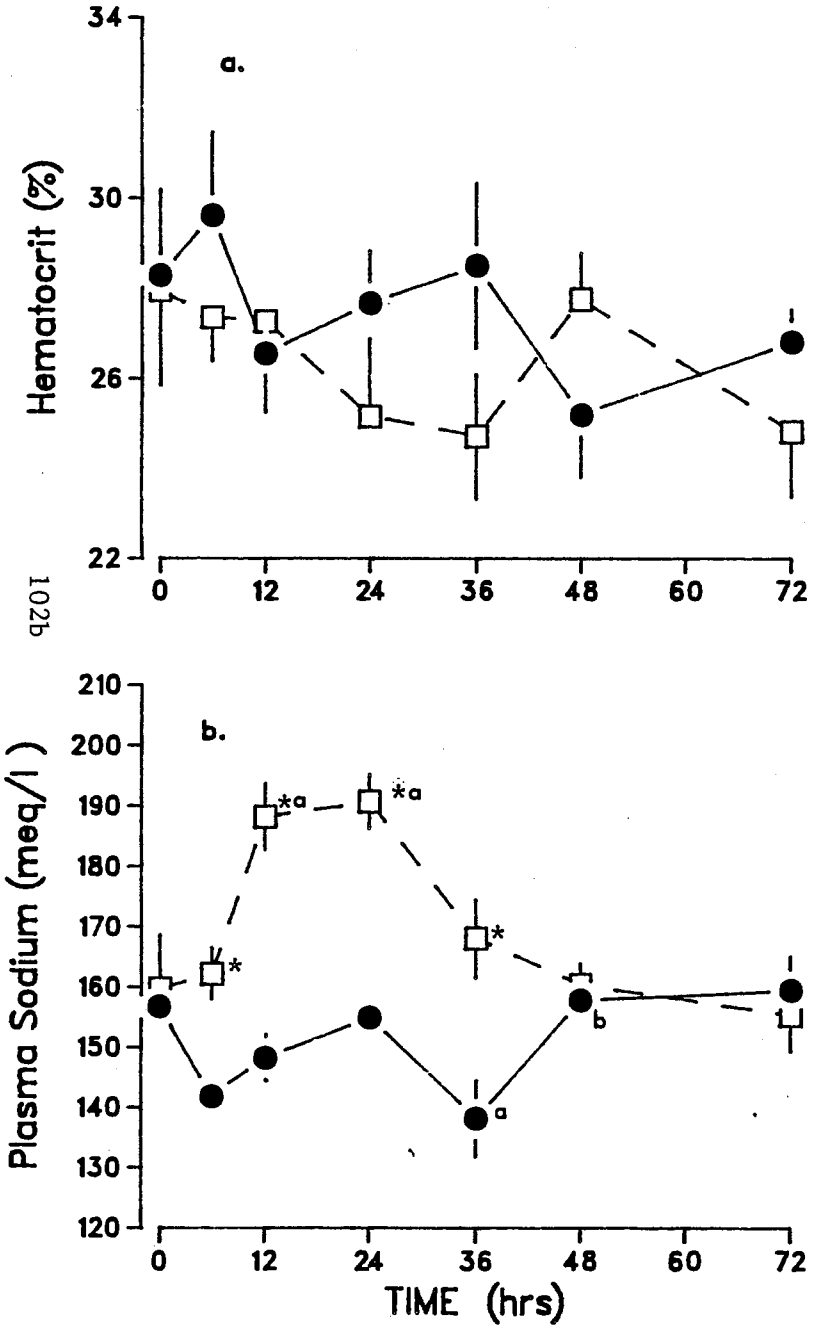

a.
Plasma Saturated FFA (umoles/l)
500
400
300
200
0
6
12
†
*f,g
b.
Plasma Unsaturated FFA (umoles/l)
200
150
100
50
0
*f
f
†e
TIME (hrs)
c.
Plasma Total FFA (umoles/l)
700
600
500
400
300
200
†
*f,g
d.
Ratio (saturated/unsaturated)
7
6
5
4
3
2
†e
†f,g
TIME (hrs)

hours after seawater entry, with the SRIF-injected group lower than the saline-injected group. Levels of these fatty acids in the SRIF-injected group were lower than pre-exposure levels at both 6 and 12 hours. The saline-injected group had lower unsaturated fatty acids than control fish at 12 hours. In examining the total FFA concentration in this experiment (Figure 19c), the control group had no differences over time, whereas the saline-injected group at 12 hours was lower than the control group as well as the levels within this group at 0 and 6 hours. The level of total FFA in the SRIF-injected group were higher than in the saline-injected group at 12 hrs. Given the lack of difference of saturates and unsaturates in the control group, it is not surprising that there were no differences in the ratio of saturated FFA/unsaturated FFA (S/U) this group (Fig. 19d). The SRIF-injected group, on the other hand, exhibited higher S/U than the saline-injected group at both 6 and 12 hours. These levels were also higher than the 0 hour level. Additionally, the 6 hour S/U was higher than the 12 hour ratio. There were no differences within the saline group nor were there any differences in S/U between between the saline-injected and control group at any time. Because this sub-experiment consisted of only 3 sample points in time, correlations were considered to be extremely difficult to interpret and, therefore, are not presented.

B. 72 Hour Seawater Exposure

There were no differences in plasma hematocrits (Figure 20a) over time in either of the two experimental groups, nor were there any differences in hematocrit between the freshwater- or seawater-exposed groups at any of the sampling periods. Plasma sodium levels in the freshwater fish (Figure 20b) demonstrated sharp declines at 6 and 36 hours, perhaps indicative of a circadian rhythm. Whether there was a decline seen at 60 hours post-exposure is unknown, but there was no indication seen in the 72 hour sampling period. While the value at 36 hrs is lower than

Figure 20: Plasma parameters in juvenile coho salmon exposed to seawater for 72 hours. All points represents (N=6-8) ± SEM. Closed circles represent freshwater controls and open squares represent fish exposed to seawater. Stars (*) indicate values significantly ($P < .05$) different than controls. Within group differences are indicated by letters such that means with the same letters are not significantly different ($P > .05$).


a.
Hematocrit (%)
34
30
26
22
0
12
24
36
48
60
72
b.
Plasma Sodium (meq/l)
210
200
190
180
170
160
150
140
130
120
TIME (hrs)
c.
Plasma GH (ng/ml)
500
450
400
350
300
250
200
150
100
50
0
d.
Plasma Glucose (mg%)
180
160
140
120
100
80
60
40

that seen at 48 hrs, the freshwater group generally maintained plasma sodium levels at approximately 155 mEq/L, considered normal for this species in freshwater. In the seawater exposed group, plasma sodium increased dramatically to 190 mEq/L by 12 hours post-exposure, was still elevated at 24 hours, then returned to freshwater (and pre-exposure) levels by 36 hours. This level was maintained until the termination of the experiment. The sodium levels at 12 and 24 hours in the seawater-exposed group were higher than all other times in this group and were also higher than all times in the freshwater group. Plasma sodium levels in the SW-exposed group were also higher than freshwater levels at 6 and 36 hours post-exposure. However, these are the times when the freshwater levels were significantly different from other freshwater times. In the seawater-exposed fish, the 6 and 36 hour plasma sodium levels were not different from the 0, 48 or 72 hour levels, nor from each other.

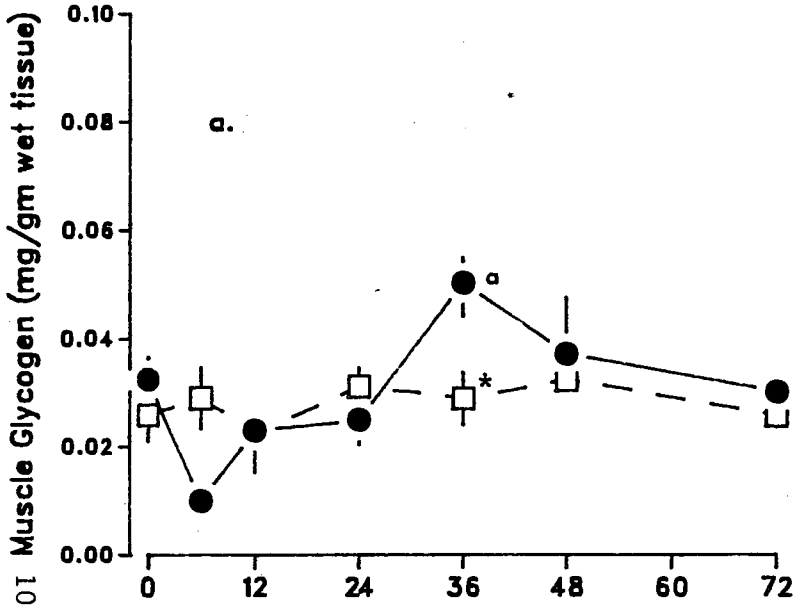
Plasma growth hormone levels (Figure 20c) in the SW-exposed fish remained at the pre-exposure level for the first 12 hours of seawater exposure, then increased approximately 2.5-fold by 24 hours. This high level was maintained for the remainder of the experiment. The levels at 36, 48, and 72 hours were higher than levels at 0 to 12 hours. Additionally, the level at 72 hours was higher than the level at 24 hours. In the FW group, there were increases in plasma GH levels at 12-24 hours and again at 72 hours. The values at these times were higher than at all other times in this group, but were not different from each other. The plasma GH levels in the SW-exposed fish were higher than in the FW fish at 24, 36, 48 and 72 hours.

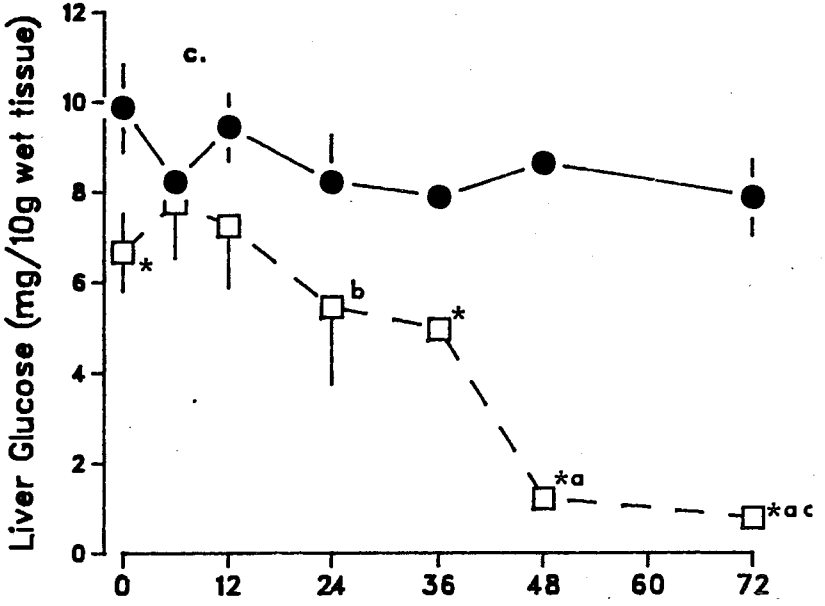
There were no differences in plasma glucose levels in the 72 hours of sampling in the FW group (Fig. 20d). In the seawater fish, there was a small rise in plasma glucose at 6 hours post-exposure which, while not different from levels at 0 or 12

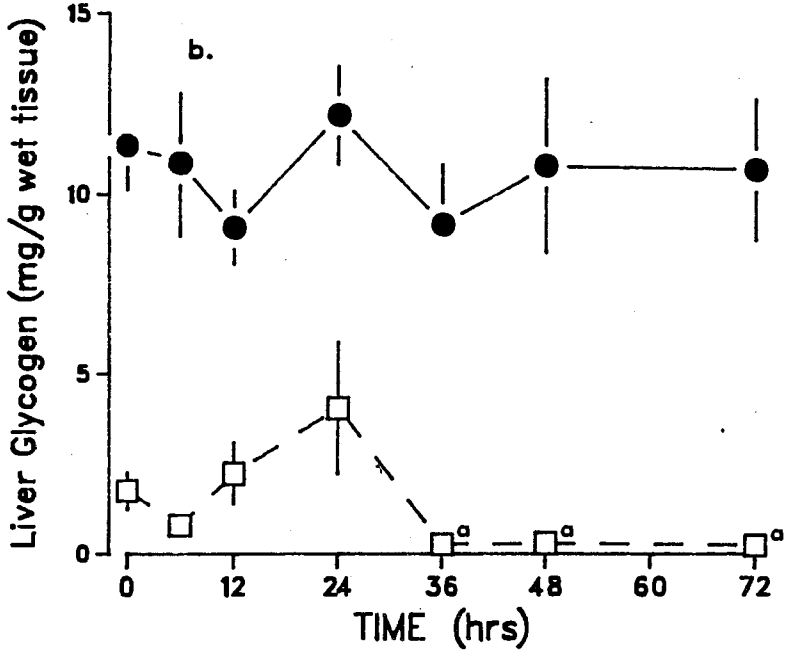
hours within the group, was significantly higher than the freshwater value at this time. Between 24 and 36 hours post-exposure there was a dramatic decline in plasma glucose concentration (Fig. 20d). This hypoglycemia persisted until termination of the experiment and the values at 36, 48 and 72 hours were all lower than values at 0-24 hours in this group. Additionally, these hypoglycemic values were lower than the freshwater values at the respective times (Fig. 20d).

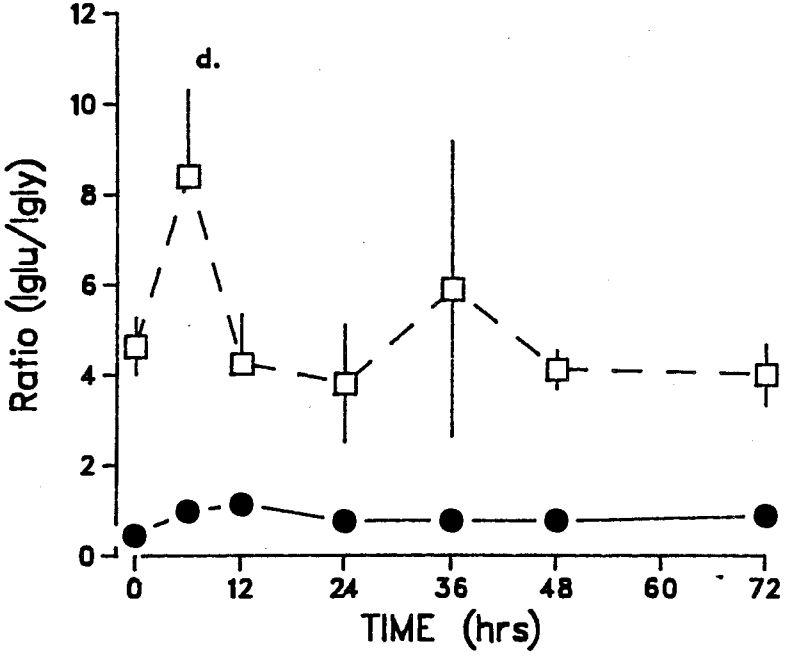
Tissue glycogen and glucose levels for the 72 hour exposure are shown in Figures 21a-c. Muscle glycogen demonstrated no changes in concentration over time in either the freshwater or the seawater-exposed groups, with the exception of the value in the freshwater group at 36 hrs, which was higher than at all other times in this group. This peak value was also higher than levels in the seawater-exposed fish at this time. Levels of glycogen in the liver tissue are shown in Figure 21b. The levels seen in the freshwater group, at time 0 (ie., prior to actual seawater exposure) were approximately 5X the level seen in the experimental ('seawater') group. The reasons for this are unknown. Assays were done on alternate groups, so assay technique does not appear to be suspect. As a result of this discrepancy, comparisons between groups were not made, and seawater within group comparisons were made with caution. The freshwater values of liver glycogen showed no change over the 72 hours of sampling. The seawater group showed an initial although nonsignificant rise from 0 to 24 hours, followed by a dramatic decline, such that the values seen at 36, 48, and 72 hours post-exposure were practically non-detectable (Figure 21b). The values seen over these three sampling periods were lower than the values seen in the initial 24 hours. Liver free glucose levels are shown in Figure 21c. Again, there were no differences within the FW group at any time over the 72 hours of sampling, suggesting that the sampling regime was not unduly stressing the animals. The

Figure 21: Tissue carbohydrates in juvenile coho salmon exposed to seawater for 72 hours. Symbols are as assigned in Figure 20. All significant differences ($P < .05$).


a.
Muscle Glycogen (mg/gm wet tissue)
0.10
0.08
0.06
0.04
0.02
0.00
0
12
24
36
48
60
72
a
*


c.
Liver Glucose (mg/10g wet tissue)
12
10
8
6
4
2
0
0
12
24
36
48
60
72
*
b
*
*a
*a c

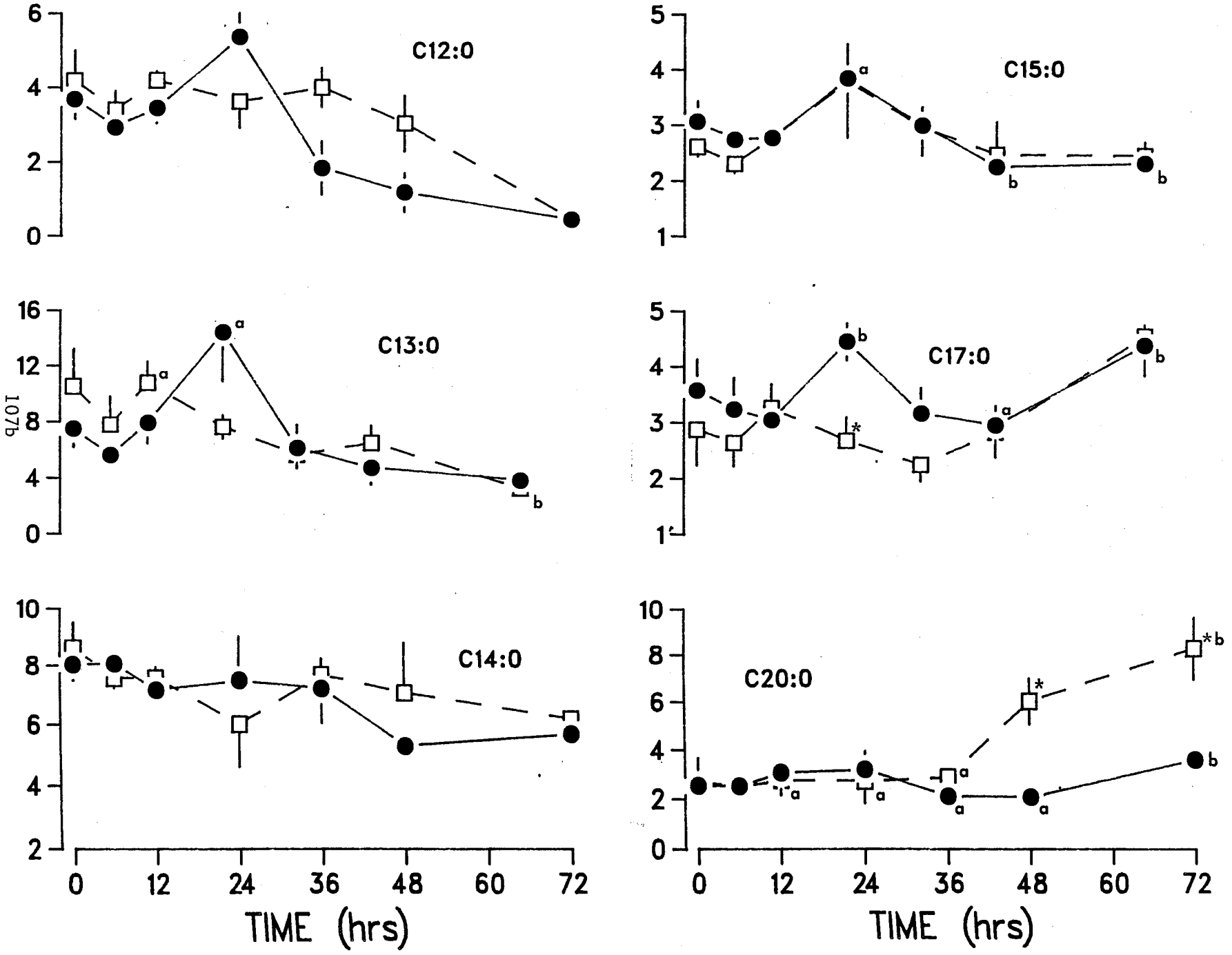

b.
Liver Glycogen (mg/g wet tissue)
15
10
5
0
0
12
24
36
48
60
72
a
a
a
TIME (hrs)

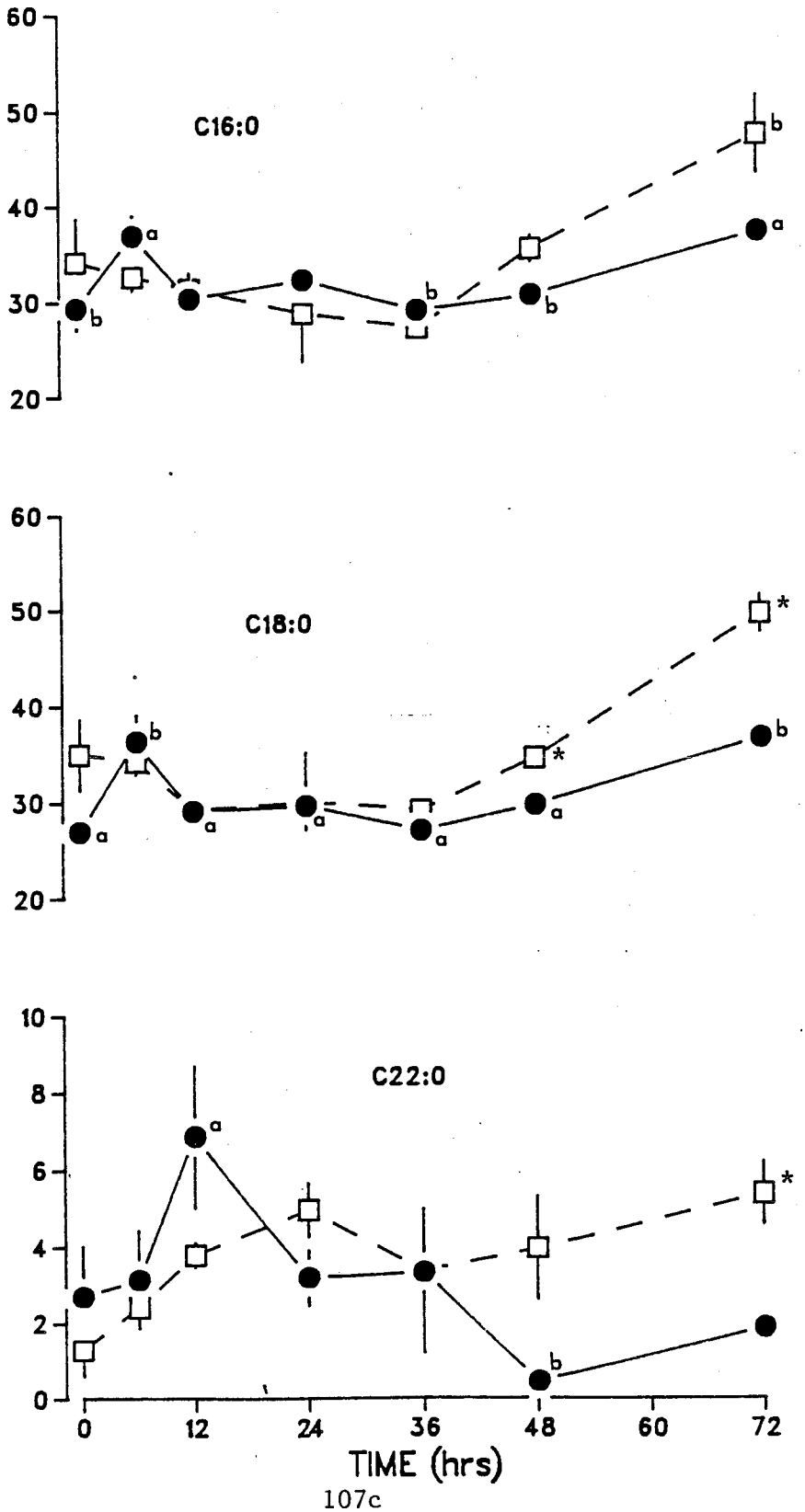

d.
Ratio (lglu/lgly)
12
10
8
6
4
2
0
0
12
24
36
48
60
72
TIME (hrs)

seawater group, however, demonstrated a decline in liver free glucose values as time progressed. The values at 48 and 72 hours were lower than those from 0 to 12 hours post-exposure. The 72 hour level was also lower than the 24 hour concentration. The pre-exposure levels in the seawater group were slightly but significantly lower than that of the freshwater group. As well, liver free glucose levels in the seawater fish were lower than the freshwater controls at 36, 48 and 72 hours. Because of the large difference in liver glycogen titers in the seawater group compared to the freshwater group, the ratio of free glucose to glycogen in the liver in the seawater also demonstrated significantly higher levels than the freshwater fish over the entire 72 hours (Fig. 21d). As seen in the other carbohydrate parameters, in the freshwater group the ratio in the liver was stable over the entire 72 hours, with no within group differences. Somewhat surprisingly, the liver free glucose/glycogen ratio in the seawater-exposed group, although much higher than seen in the freshwater control group, were also very stable over the course of the sampling.

The plasma concentrations of the individual FFA are shown in Figures 22-24, with the saturated FFAs in Figure 22. In freshwater, there was a general decrease in lauric acid (C12:0) levels over the 72 hours of the study, with the exception of the 24 hour sampling. Plasma levels at this time were higher than at 6,36,48 and 72 hours. Otherwise, the 72 hour level was lower than levels from 0 to 24 hours. The 48 hour level was lower than both 0 and 24 hours. In seawater, the levels were maintained at a relatively stable concentration, other than the 72 hour value, lower than all other times in this group. Between groups, there were no differences at anytime. Tridecanoic acid (C13:0) also demonstrated a sharp peak in the freshwater group at 24 hours, which was higher than all other sampling times within this group. There were no other differences within the freshwater group,

Figure 22: Plasma saturated free fatty acids in juvenile coho salmon exposed to seawater for 72 hours. Symbols are as assigned in Figure 20. All significant differences ($P < .05$).


C12:0
C15:0
C13:0
C17:0
C14:0
C20:0
10Tb
TIME (hrs)
TIME (hrs)


C16:0
C18:0
C22:0
60
50
40
30
20
10
8
6
4
2
0
0
12
24
36
48
60
72
TIME (hrs)
a
b
*

although the general trend was declining levels. In seawater, there was no peak at 24 hours, and the 72 hour level was lower than the 12 hour level. There were no differences between the two groups at any time.

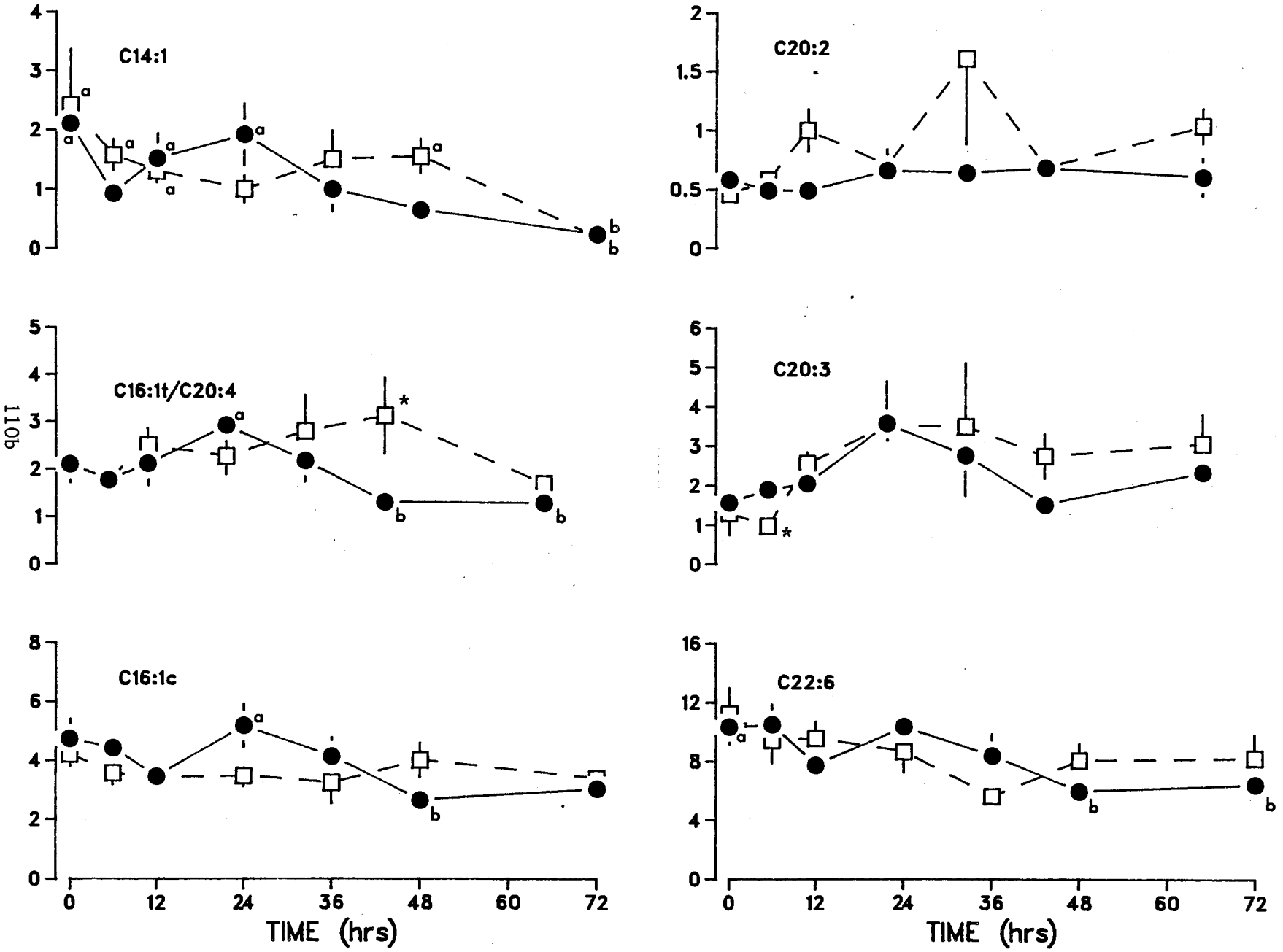
There were no differences in plasma myristic acid (C14:0) levels within or between groups over the entire 72 hours of the experiment. In both the freshwater and seawater groups there was a peak of pentadecanoic acid (C15:0) at 24 hours post-exposure. In the FW group, this peak was higher than levels seen at 48 and 72 hours. There were no differences between the two groups at any time. Heptadecanoic acid (C17:0), shown in Figure 22, also exhibited a rise over the time course of the experiment. In the freshwater fish, the levels at both 24 and 72 hours were higher than that seen at 48 hours. There were no differences within the seawater group, nor were there differences between the two groups at any time with the exception of the 24 hour sample. The plasma levels of arachidic acid (C20:0)(Fig. 22) in freshwater were higher at 72 hours than at 36 or 48 hours. The seawater fish exhibited a large increase in plasma titer of this acid post-36 hours, and the levels seen at 72 hours were higher than from 0-24 hours. As well, the levels at 48 and 72 hours were higher in the seawater group than in the freshwater group at these times.

Palmitic acid (C16:0) (Fig. 22) demonstrated a general increase in plasma concentration in the freshwater fish over the experiment. The levels at 6 and 72 hours were higher than those seen at 0, 36 and 48 hours. In the seawater-exposed fish, the 72 hour levels were higher than all other times. Although the SW group had a trend towards higher levels than the FW fish, there were no significant differences by the end of the sampling regime. In freshwater, plasma levels of stearic acid (C18:0)(Fig. 22) were higher at 6 and 72 hours than all other sampling times in this group. In the seawater fish, the 72 hour level was higher than all other times. Additionally, the seawater-exposed fish had

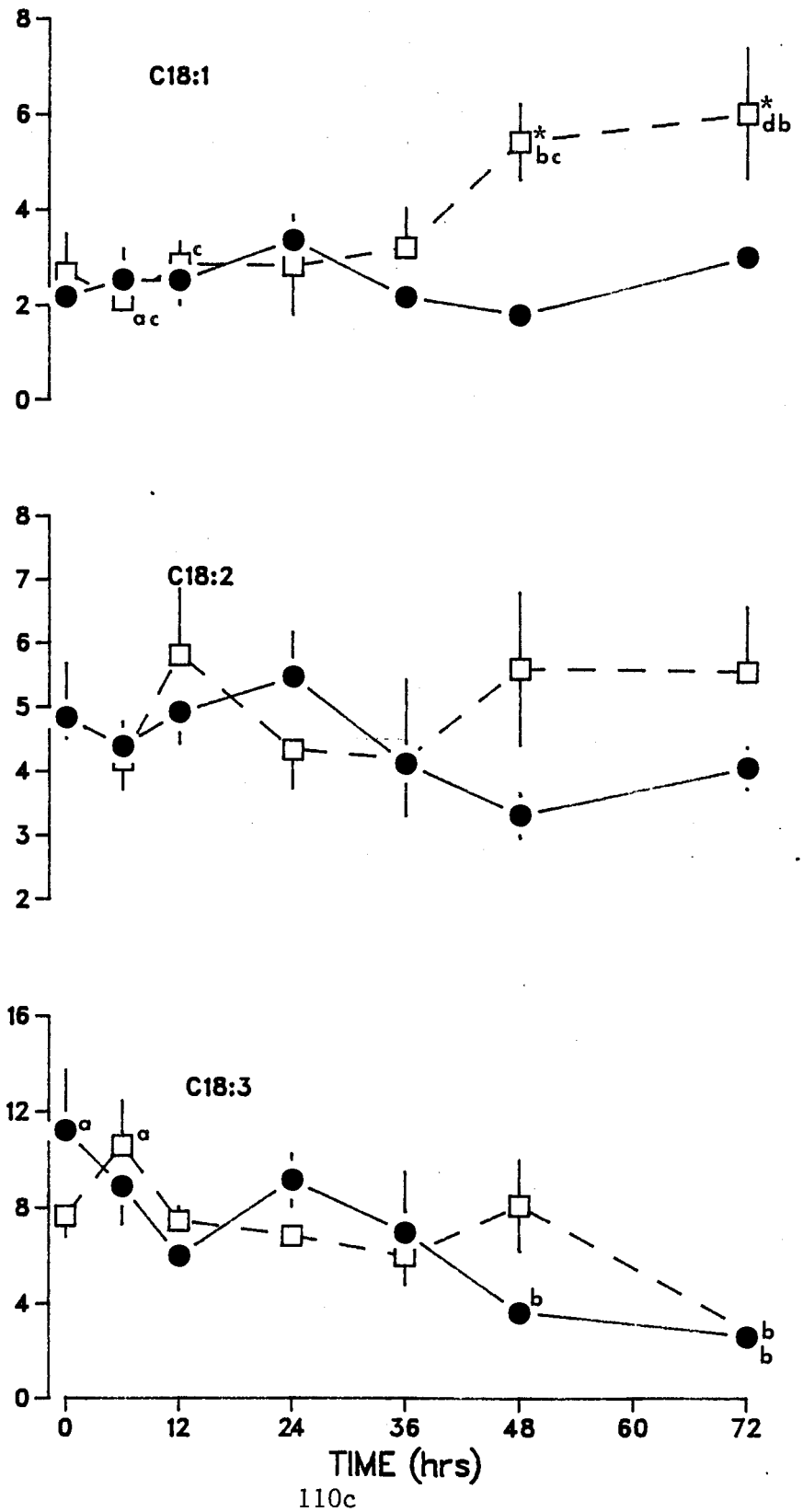
higher plasma levels of this fatty acid than the freshwater fish at 48 and 72 hours. Behenic acid (C22:0)(Figure 22) showed a peak at 12 hours in the freshwater group which was significant only from the 48 hour sample in this group. The seawater-exposed fish did not show any within group differences at any time in the 72 hours. The level in the seawater group at 72 hours was, however, higher than the level seen in the freshwater group at this time. In general, plasma levels of saturated FFA in both groups seemed to demonstrate two patterns. Those fatty acids composed of 15 carbons or less seemed to decline over the course of the experiment. On the other hand, those saturated FFA of 16 carbons or more demonstrated a general increase over the sampling regime, especially post-36 hours.

The individual unsaturated FFAs are shown in Figure 23. In freshwater, there was a general decline in plasma levels of myristoleic acid (C14:1), with the 72 hour level lower than levels at 0,12 and 24 hours. The 48 hour level was lower than both 0 and 24 hours, and the 36 hour level was lower than only the 0-hour concentration. In seawater, only the 72 hour level was lower (than 0, 6, 12 and 48 hours). There were no differences between the two groups. Plasma levels of the two sixteen carbon monounsaturates are also shown in Figure 23. Palmitelaidic acid, the C16:1*trans* molecule, exhibited a slight peak at 24 hours in the freshwater group. This peak was higher than levels seen at 48 and 72 hours. There were no differences within the seawater exposed fish over the 72 hour sampling regime. Between groups, the plasma concentration in the seawater group at 48 hours was higher than in the freshwater fish. Palmitoleic acid, the C16:1*cis* molecule, also showed a slight peak at 24 hours in the freshwater fish, which was absent in the seawater group. This peak was higher than values seen at 48 hours in this group. There again were no differences in plasma levels of this acid in the seawater group over 72 hours, nor were there any differences

Figure 23: Plasma unsaturated free fatty acids in juvenile coho salmon exposed to seawater for 72 hours. Symbols are as assigned in Figure 20. All significant differences ($P < .05$).



110b

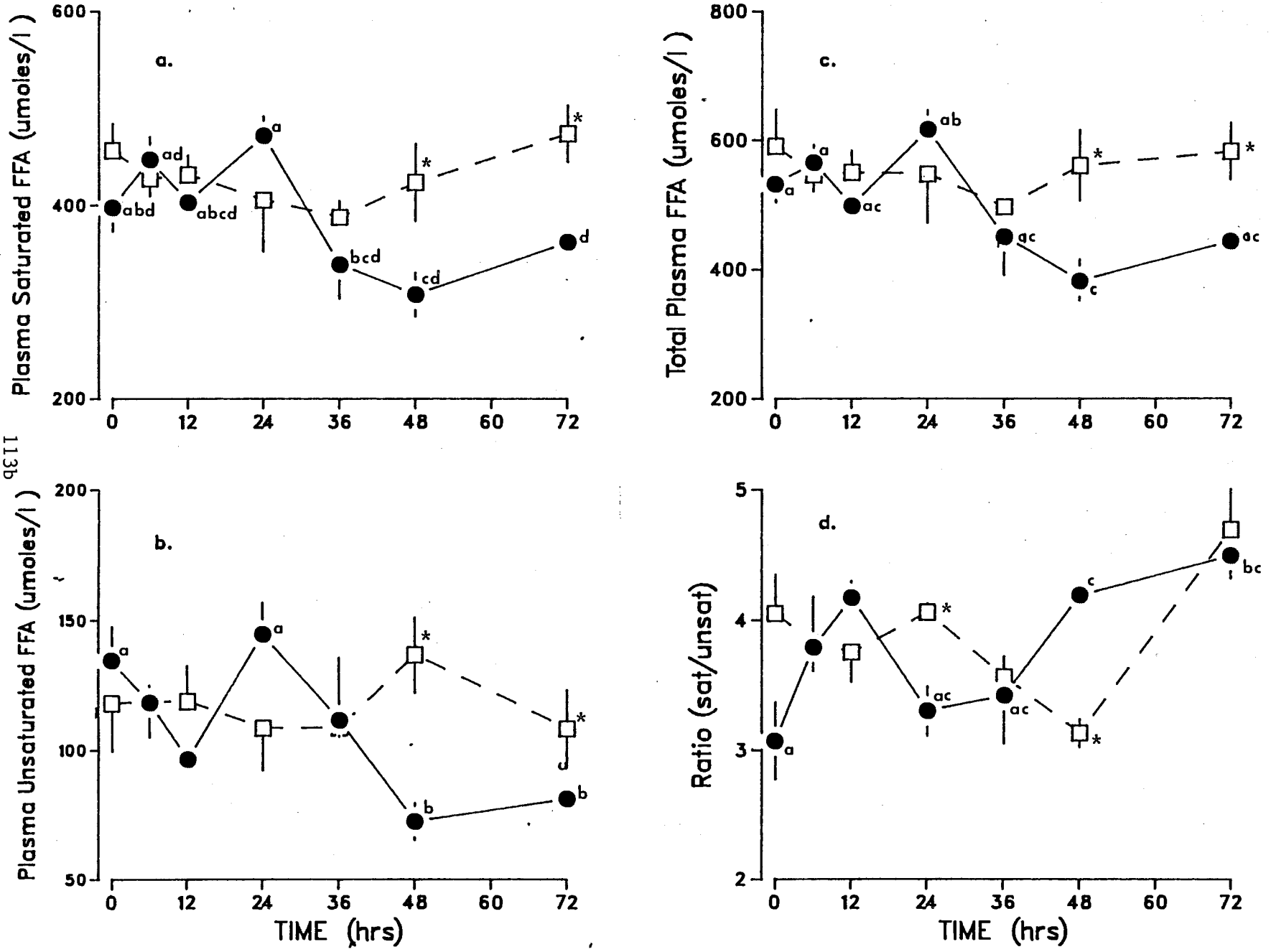

C18:1
C18:2
C18:3
TIME (hrs)
0
12
24
36
48
60
72

between the two groups. Plasma concentrations of eicosadienoic acid (C20:2) (Fig. 24) showed no differences within either group over the sampling period, although the SW-group had a general increasing trend. There were also no differences between the two groups at any time. Plasma levels of eicosatrienoic acid (C20:3)(Fig. 23) also showed no within group differences in either the FW- or the SW-group. There plasma level in the SW-group at 6 hours post-exposure was lower than in the FW fish at this time. This was the only between group difference. In SW, the plasma values again demonstrated a general increase over the sampling regime, similar to the saturated FFA with more than 16 carbons. In freshwater, the 48 and 72 hour concentrations of docosahexaenoic acid (C22:6)(Fig. 23) were lower than the 0-hour level. In the seawater group, there were no differences at any time. There were also no differences between the freshwater and seawater groups at any time.

Both oleic and linoleic acid (C18:1 and C18:2, respectively) (Fig. 23) demonstrated slight rises from 36 to 72 hours, similar to those seen in the high (> 16) carbon saturated fatty acids. The values of oleic acid at 48 and 72 hours were higher in the seawater group than in the freshwater controls. The levels in this group at 48 hours were higher than at 6 hours, whereas the 72 hour levels were higher than both 6 and 12 hours in this group. There were no differences within the freshwater fish. While the pattern of linoleic concentrations are similar to those of oleic acid, there were no differences either within or between the two groups. Plasma values of linolenic acid, C18:3, were characterised by a decline over time. In the freshwater group, both the 48 and 72 hour levels were lower than the 0-hour concentration. In the seawater group, the 72 hour level was lower than the 6 hour level. There were no differences between the two groups at any sampling time.

Plasma total FFA concentrations for the 72 hour SW exposure are shown in Figure 24a-d. The saturated FFA levels (Fig. 24a) were significantly higher in the SW group than in the FW group at 48 and 72 hours, although there were no within group differences in the SW group. The freshwater fish exhibited declines over the 72 hours of sampling, such that the levels of saturated FFA in the plasma at 36 hours were lower than levels at 6 and 24 hrs and levels at 48 hrs were lower than the levels seen at 0, 6, and 24 hrs, but levels at 72 hours were only significantly lower than the level of saturated FFA seen at 24 hrs. Plasma levels of unsaturated FFA (Fig. 24b) show similar trends as seen in the saturated FFA. The FW group showed declines over the sampling period, with plasma levels at 48 and 72 hours lower than levels seen at both 0 and 24 hrs. The SW group again exhibited no within-group differences over time, but plasma levels of unsaturated FFA in the SW group were higher than in the FW fish at both 48 and 72 hrs, as seen in the saturated FFA. The total FFA in the plasma (Fig. 24c) demonstrated the same results as seen for the two classes of FFA: that is, levels in the SW group demonstrated no changes over the 72 hours of sampling whereas the FW group showed a decline. Again, the SW fish at 48 and 72 hours had higher total FFA levels than the FW fish at these times. Levels within the FW group at 36, 48, and 72 hours were lower than the level seen at 24 hrs, and the level at 48 hrs was also lower than that seen at 0 and 6 hours. All three components (ie., saturated, unsaturated, and total FFA) showed a fairly steady decline in plasma titers in the FW group except for a peak at 24 hours. This peak at 24 hours in the FW group is also seen in the ratio of saturated to unsaturated FFA (S/U)(Figure 24d). The S/U in the FW group at 48 and 72 hours was higher than the ratios seen at 0 hrs, and the 72 hour value was also higher than those at 24 and 36 hours in this group. There were no differences within the SW group, as seen in the previous plots. Between groups, the S/U ratio in the SW group was significantly higher

Figure 24: Plasma FFA totals and the satFFA/unsatFFA ratio in juvenile coho salmon exposed to seawater for 72 hours. Symbols and letters are as assigned in Figure 20. Values for 24a, b, and c are expressed as μmoles FFA/L plasma. All significant differences ($P < .05$).


a.
Plasma Saturated FFA (umoles/l)
600
400
200
abd
ad
abcd
a
bcd
cd
d
*
0
12
24
36
48
60
72
b.
Plasma Unsaturated FFA (umoles/l)
200
150
100
50
a
b
TIME (hrs)
c.
Total Plasma FFA (umoles/l)
800
600
400
200
a
ab
ac
c
d.
Ratio (sat/unsat)
5
4
3
2
bc

than the FW group at 24 hrs post-exposure, whereas the ratio at 48 hours was lower than the ratio seen in the FW group at this time.

Table 6 shows the correlation coeffecients between the two treatment groups, utilizing the major parameters measured in this experiment. Not all of the plasma FFA were used in this table, only the ones which have been attributed importance in either metabolism or structural functions in fish in the literature. In the freshwater controls, plasma Na^+ levels correlated positively with free glucose levels in the liver. Plasma Na^+ also correlated positively with plasma unsaturated fatty acid concentrations and negatively with the sat/unsat ratio. The saturated fatty acids shared a strong correlation with the unsaturated fatty acids but only the saturated fatty acids were correlated with muscle glycogen levels. The concentration of glycogen in the liver correlated postively with muscle glycogen and plasma glucose levels. Conversely, the liver glycogen levels were negatively correlated with both weight and length.

In the seawater group, plasma Na^+ correlated positively with plasma rather than liver glucose. Plasma Na^+ also correlated, negatively, with circulating levels of saturated fatty acids. Plasma levels of growth hormone showed strong negative correlations with both plasma and liver glucose, and these two glucose compartments also demonstrated a strong correlation with each other ($n=43; r=.7221$). Liver free glucose also correlated with the liver glycogen levels. Plasma levels of saturated and unsaturated fatty acids were positively correlated.

In seawater, plasma growth hormone showed strong correlations with many of the individual free fatty acids, both saturated and unsaturated. Of particular interest were the very strong correlations with linolenic, oleic, palmitic and arachidic acids. Plasma Na^+ values showed negative correlations with oleic,

Table 6: Correlations between parameters of the 72 hour seawater adaptation. The abbreviations are as described in the text. The correlations are one-tailed where single stars (*) represent ($P \leq .01$) and double stars (**) indicate ($P \leq .001$). See text for detailed discussion.

A. Freshwater

	Cond. Factor	Hemato	Plasma GH	Plasma Sodium	Plasma Glucose	Liver Glycogen	Liver Glucose	Ratio Glu/Gly	Muscle Glycogen
C. Fact.	1.0000	.1131	-.2014	.1069	.0094	.2386	-.3468	-.2677	.2786
Hemato		1.0000	-.0343	.1965	.3363	.4635*	.0100	-.1781	.2345
GH			1.0000	-.1162	-.1799	-.0821	-.0163	.0088	-.2253
P-Sodium				1.0000	.2864	.1471	.3556	-.1465	-.1015
P-Glucose					1.0000	.3398	-.1513	-.2358	-.0651
L-Glycogen						1.0000	-.1676	-.8137**	.3672
L-Glucose							1.0000	.3778	-.0865
Ratio Glu/Gly								1.0000	-.3298
M-Glycogen									1.0000

	Plasma C16:0	Plasma C22:6	Plasma C18:0	Plasma C18:3	Plasma satFFA	Plasma unsatFFA	Plasma Total FFA	Ratio sat/unsat
C. Fact.	-.2245	.0421	-.1880	-.1224	-.0729	-.0242	-.0559	-.1188
Hemato	.0351	.4038	-.2475	.1758	.1957	.4075	.2760	-.3537
GH	.2496	-.0498	.3380	.0304	.2965	.0505	.2246	.1578
P-Sodium	-.0842	.2724	-.2843	.2889	.1496	.3396	.2339	-.3855
P-Glucose	.0310	.1106	-.0496	.2150	-.1297	.1019	-.0705	-.1403
L-Glycogen	-.0656	.2225	-.3480	-.0738	.0143	.2198	.0464	-.2045
L-Glucose	-.1531	.0090	-.2295	.2140	-.0111	.0949	.0267	-.1113
Ratio Glu/Gly	.0111	-.2723	.2057	.0716	-.0485	-.1840	-.0934	.2221
P-C16:0	1.0000	.1714	.8333**	-.1648	.6513**	.0335	.4773*	.3109
P-C22:6		1.0000	-.1508	.6453**	.6353**	.9151**	.8199**	-.7857**
P-C18:0			1.0000	-.2027	.4452*	-.2392	.2475	.4699*
P-C18:3				1.0000	.1910	.8002**	.4633*	-.8159**
satFFA					1.0000	.5873**	-.9418**	-.2432
unsatFFA						1.0000	.8190**	-.8887**
Total FFA							1.0000	-.5317*
Ratio S/U								1.0000

B. Seawater

	Cond. Factor	Hemato	Plasma GH	Plasma Sodium	Plasma Glucose	Liver Glycogen	Liver Glucose	Ratio Glu/Gly	Muscle Glycogen
C. Fact.	1.0000	-.2384	.0390	-.1680	-.0082	.4035*	.0320	-.3034	.2485
Hemato		1.0000	-.3157	-.0819	.1617	-.0977	.1549	.2727	.1183
GH			1.0000	-.2788	-.6473**	-.2648	-.6470**	-.0802	.0968
P-Sodium				1.0000	.3824*	.2677	.2390	-.3568	.0072
P-Glucose					1.0000	.2433	.7221**	.1260	.0221
L-Glycogen						1.0000	.5366**	-.4104*	.2202
L-Glucose							1.0000	.0182	.1684
Ratio Glu/Gly								1.0000	.0733
M-Glycogen									1.0000

	Plasma C16:0	Plasma C22:6	Plasma C18:0	Plasma C18:3	Plasma satFFA	Plasma unsatFFA	Plasma Total FFA	Ratio sat/unsat
C. Fact.	.4664*	.6203**	.3009	.1724	.5135*	.5913**	.5923**	-.2538
Hemato	-.0640	.1229	-.2509	.2663	-.1104	.2677	.0246	-.4667
GH	.4711*	-.2622	.5810*	-.4787*	.3617	-.0288	.3394	.3628
P-Sodium	-.4901*	.0884	-.6250**	.2084	-.3741	-.0100	-.3231	-.2826
P-Glucose	-.4239	.3601	-.5147*	-.5091*	-.3357	.1212	-.2225	-.2794
L-Glycogen	-.3747	.0502	-.4755*	.0005	-.3673	-.0190	-.1230	-.1293
L-Glucose	-.3671	.1576	-.4582*	.2590	-.2645	-.0510	-.1972	-.1427
Ratio Glu/Gly	-.2222	-.2620	-.0736	.1661	-.1777	-.1569	-.2767	-.0327
P-C16:0	1.0000	.3819	.8988**	-.2878	.9139**	.3505	.8542**	.1803
P-C22:6		1.0000	.0989	.4824*	.5100*	.8326**	.6869**	-.5232*
P-C18:0			1.0000	-.4003	.8097**	.0336	.6598**	.4872*
P-C18:3				1.0000	-.1708	.5819**	.0100	-.6570**
satFFA					1.0000	.5106*	.9571**	.1019
unsatFFA						1.0000	.7271**	-.7143**
Total FFA							1.0000	-.1491
Ratio S/U								1.0000

stearic, palmitic and arachidic acids. Plasma glucose had positive correlations linolenic and docosahexaenoic acids and positive correlations with palmitic, oleic, stearic and arachidic acids.

The tissue levels of glycogen in the seawater group also demonstrated correlations to plasma levels of some of the fatty acids. Thus, liver glycogen had negative correlations with both stearic and palmitic acids. Liver free glucose had negative correlations with oleic, stearic and arachidic acids. Muscle glycogen levels were not correlated with plasma levels of any of the individual fatty acids in the seawater group.

In the freshwater controls, plasma growth hormone also demonstrated positive correlations with plasma values of oleic and stearic acids, but no correlations with any other fatty acids. At the tissue level, liver glycogen was negatively correlated with plasma stearic acid. Muscle glycogen in the freshwater fish, unlike the seawater group, showed correlations, all negative, with plasma oleic, stearic and arachidic acids levels.

DISCUSSION

Adaptation of the anadromous salmon to seawater may be separated into two components or phases (Houston, 1959;Hickman and Trump, 1969; LeRay *et al.*, 1981;Raffin, 1986). The initial component is considered to be essentially a passive (ie., little or no direct energy costs) and reversible phase, characterized by increases in plasma electrolyte levels, redistribution of tissue water volumes, possible uptake of various inorganic ions by soft tissues and bone, and variations in membrane permeabilities of surface tissues (Houston, 1959). He refers to these actions as a type of 'osmoregulatory buffer system' which acts to minimize the rates and/or magnitudes of the changes in electrolyte composition during this 'adjustive' phase. The duration of this initial 'crisis' phase is dependent on both the species and upon the degree of smoltification (including size) of the fish. In 2-year-old rainbow trout, LeRay *et al.* (1981) reported a duration of approximately 30 hours, whereas Clarke and Blackburn (1977) and Blackburn and Clarke (1987) suggest < 24 hours is characteristic for *Oncorhysnchus* spp. smolts. Hogstrand and Haux (1985) found a similar time frame for *Salmo trutta* smolts. LeRay *et al.* (1981) also failed to note any increase in respiratory rates of the gill tissue during this initial 'crisis' period, providing further evidence of the passive nature of this phase. Eddy (1982) noted that, immediately upon entry to seawater, juvenile rainbow trout increased their drinking rates from 0.5 mL/Kg hour^{-1} to 25 mL/Kg hour^{-1}. This high rate of drinking continued for some hours before dropping to levels characteristic of marine fish, approximately 5.0 mL/Kg hour^{-1}. In addition to increased drinking rates in this initial phase of seawater adaptation, euryhaline fish also reduce urine output levels. Holmes (1961) found that levels of urine flow were 1-5% of the freshwater levels in salmonids within 18 hours of seawater transfer. This reduction is achieved, in part, by reducing both

the number of filtering glomeruli as well as the blood flow to the glomeruli (Colville *et al.*, 1983;Gray and Brown, 1987). These responses act to compensate for the osmotic water loss to the hyperosmotic environment during the adjustive phase. Again, these responses can be considered relatively passive.

The second phase proposed by Houston is characterised by energy-consuming activities, which will act to restore circulating electrolyte levels to the homeostatic state, usually somewhat slightly above the values seen in the freshwater fish. Features of this phase include chloride cell hyperplasia and differentiation, induction of high Na^+,K^+-ATPase enzyme levels, etc. Chloride cells, or Keys-Wilmer cells, are located on the gill primary lamellae and secrete large amounts of Na^+ and Cl^- ions in marine fish, primarily via the Na^+,K^+-ATPase enzyme systems (Foskett *et al.*, 1983). The number of chloride cells has been found to be directly related to external salinity in a number of euryhaline species, although not in coho salmon smolts (cf. Folmar and Dickhoff, 1980). Boeuf *et al.* (1978) did, however, find direct effects of external salinity on the magnitudes of increases in Na^+,K^+-ATPase activity in the gills of coho salmon smolts. Nonnotte *et al.* (1987) further noted increases in intestinal Na^+,K^+-ATPase activity in rainbow trout during the regulatory phase of seawater adaptation (ie., after 24 hours). Also associated with the regulatory phase, LeRay *et al.* (1981) noted significant declines in gill ATP/ADP ratios and energy charges, indicative of energetic metabolic responses. It is important to note that while many indices of the parr-smolt transformation (eg., plasma cortisol, T_4, and GH levels, increased gill Na^+,K^+-ATPase activity, increased salinity tolerance and preference) actually seem to peak prior to downstream migration and seawater entry (Hoar, 1976;Bern, 1978;Folmar and Dickhoff, 1980;Wedemeyer, 1980), a truly smolted fish is able to tolerate and regulate in seawater.

Results from both seawater transfer experiments (24 hour and 72 hour) demonstrated that transfer of coho salmon smolts to seawater led to dramatic and sustained (> 72 hours) elevations in plasma growth hormone concentrations. The increase in plasma titers of GH was probably the result of increased synthesis and release, as opposed to a decrease in uptake. Not only does the magnitude and duration of the elevated levels suggest this, but, as mentioned previously, several authors have histologically demonstrated increased activity (both synthetic and secretory) of pituitary somatotroph cells in salmon smolts transferred to seawater (Komourdjian *et al.*, 1976;Nagahama *et al.*, 1977;Nishioka *et al.*, 1982). Increased synthetic activity and/or secretion have also been noted in a non-salmonid euryhaline fish exposed to high external salinities, the nine-spined stickleback, *Pungitius pungitius*, (Benjamin, 1978). While pre-exposure plasma GH levels in the seawater groups were the same in both experiments (≃ 100 ng/ml), the elevation in the first experiment (May, 1983) was seen between 6 and 12 hours post-transfer whereas it was somewhat delayed (12-24 hours post-transfer) in the second experiment (June, 1984). Recall that both experiments had SW gradually introduced, such that full strength salinity (≃ 33 ppt) was not reached for 2-3 hrs. It may be that the one month difference in transfer timing was reflected in the degree of smoltification. Although both experiments utilized yearling fish that appeared externally to be fully smolted (silvery appearance, dark fin margins, etc.), it is well documented that salinity tolerance increases as smoltification proceeds (Komourdjian *et al.*, 1976;Boeuf *et al.*, 1978;Clarke *et al.*, 1977;Nagahama *et al.*, 1982;Saunders *et al.*, 1983) and decreases as desmoltification occurs (Conte and Wagner, 1965;Folmar *et al.*, 1982; Mahnken *et al.*, 1982). Johnston (1983) further noted that this cycle in tolerance could be seen in gill Na^+,K^+-ATPase activity in Atlantic salmon smolts and desmolts. Additionally, as mentioned previously, the peak in seawater tolerance and

regulatory ability usually occurs well before the smolts exhibit peak migratory activity (Folmar and Dickhoff, 1981;Hogstrand and Haux, 1985). Boeuf and Harache (1982) noted that the length of the adjustive and regulatory phases was dependent upon the level of Na^+,K^+-ATPase activity in the gills of the fish prior to seawater transfer (ie., upon the degree of smoltification). Thus, the delay of 6 hours in the post-transfer increase seen in the second experiment may be indicative of a less developed seawater tolerance (leading to an increase in the length of the initial, adjustive phase) in this group of fish. However, it is also important to recognize that although both groups of coho used in the experiments were obtained from the same hatchery, they represent different year classes and different genetic backgrounds and, therefore, different rates and/or degrees of smoltification or desmoltification.

Bolton *et al.* (1987a) demonstrated that the transfer of mature chum salmon from seawater to freshwater had no effect on plasma GH levels at 1,3 or 7 days post-transfer, which suggests that the rise in plasma GH seen in the reverse transfer (FW to SW) was not the result of simple stress responses. Additionally, transfer of immature coho salmon from seawater to freshwater (Sweeting *et al.*, unpub.) also failed to induce rises in plasma titers of GH at 1, 2, 4, 8, 24, 48, or or 72 hours post-transfer. Indeed, at 48 hours the plasma GH levels in the FW group of this experiment (SW > FW) were significantly lower than in the SW control group (64.31 ± 3.94 vs 162.63 ± 23.86; P=.002). Although both groups in this reverse transfer experiment exhibited slight increases in plasma GH levels at 0.5 hours, the SW controls did not demonstrate any effects of sampling stress on plasma GH levels. There were also no indications of sampling-stress induced elevations of plasma GH in the FW control groups of either of the FW to SW transfer experiments. It seems apparent, then, that due to the duration and magnitude of elevations seen in both of the

seawater experiments (≃ 4-6 fold), elevated plasma growth hormone may be a standard physiological response to increased environmental salinity. Elevations in plasma cortisol following seawater transfer of salmon smolts have also been reported (Hirano, 1969;Ball *et al.*, 1971;Forrest *et al.*, 1973;Redding *et al.*, 1984a,b). This hormone, however, appears to be involved in the initial phase of adaptation, as the elevated plasma titers occur very rapidly. Both Strange and Schreck (1980) and Redding *et al.* (1984a,b) noted peaks in plasma cortisol within 2 hrs of transfer of coho smolts to SW. Nichols and Weisbart (1985) reported similar timing of cortisol peaks in Atlantic salmon transferred to seawater. In all of these studies, the plasma cortisol levels had returned to control values by 6-10 hours post-transfer. Redding *et al.* (1984b) suggested that the role of cortisol in SW adaptation of coho may be a stress response, enhancing general metabolic requirements. In support of this proposal, Assem and Hanke (1981) found that transfer of the euryhaline tilapia from 3.5% SW to FW also led to a transient (6 hr) elevation in plasma cortisol titers. While I have no knowledge of any reports of GH activity in non-salmonid euryhaline fish following transfer to hypersaline environments, it seems that, in smolts of anadromous salmonids, the 6-20 hour delay in the rise of GH, as well as the duration of the elevated levels, suggests that somatotropin has a role in the regulatory, as opposed to the adjustive, phase of SW adaptation. Cortisol, on the other hand, appears to be more important in the initial, passive phase.

Somatostatin was obviously very effective in lowering plasma growth hormone levels well below both the pre-exposure titer and the subsequent rises seen in the saline-injected group. While there was no histological support, this reduction in plasma GH titers was presumed to be an inhibition of release from the pituitary somatotroph cells, as, to my knowledge, this is the

only action of somatostatin on GH. These results agree with the actions seen in numerous mammalian studies. While this was the first report of the action of somatostatin in salmonids, its' effectiveness had been demonstrated in several teleosts, including tilapia, *Oreochromis mossambicus* , (Fryer *et al.* , 1979) and the sailfin molly, *Poecilia latipinna* (Batten and Wigham, 1984) in *in vitro* studies, and in the goldfish, *Carassius auratus*, *in vivo* (Cook and Peter, 1984). That there were no mortalities in the SRIF group by the 12 hour sampling period lends further credence to the hypothesis that GH is involved in the regulatory phase of adaptation and that other compounds, such as cortisol, may be primarily involved in the short-term adjustive response.

In the 72-hour seawater experiment, plasma Na^+ concentrations were being regulated (that is, no longer increasing) by 24 hours post-exposure and were down to freshwater or control levels by 36 hours post-exposure. Thus, the regulation of plasma Na^+ seems somewhat delayed in this experiment, according to the saltwater challenge test of Blackburn and Clarke, even though the fish appeared to be fully capable of successful adaptation to seawater. Plasma Na^+ levels from the first seawater experiment were also analysed, with the plasma remaining after other assays were completed. As a result the sample sizes for each time period are small (n=2-5). Regardless, the results demonstrated return of plasma Na^+ titers to freshwater levels by the 24 hour sampling time (192.00 mEq/L versus 171.9 mEq/L at 12 and 24 hours,respectively), again suggesting the fish from this first experiment had increased seawater tolerance over the fish from the second experiment. Despite the small sample sizes in these measurements, there was a strong and significant (P=.014) correlation between plasma GH and Na^+ levels in this initial experiment (n=13;r=.6043). Although there were no correlations between body weight and plasma GH or body length and plasma GH,

nor between body weight and plasma Na^+ or body length and plasma Na^+, controlling for weight increased the initial correlation between GH and Na^+ (n=10;r=.6633;P=.009), as did controlling for body length (n=10;r=.6372;P=.013). Controlling for both weight and length further improved the correlation (n=9;r=.6732;P=.012). These results suggest that, in this first study, there was a direct correlation between the amount of GH secreted and the level of Na^+ ions which enter the circulation. Additionally, a strong aspect of body size seems apparent. According to the literature, smaller fish should have a greater influx of Na^+ ions upon SW entry (due to large surface area/volume ratios), as discussed in Chapter 1. Grau *et al.* (1986) reported that juvenile fish also have higher plasma levels of GH than adults, as is the case in mammals (Turner and Bagnara, 1976). However, correlations between body size and either plasma GH or Na^+ were not seen in either of my exposure experiments, probably a reflection of the narrow size range used in the experiments, done to avoid just these types of interactions. Hogstrand and Haux (1985) also noted no correlation between body size and plasma Na^+ levels in *S. trutta* transferred to 20 ppt. seawater. Bolton *et al.* (1987a) noted a significant negative correlation between plasma Na^+ and body weight in rainbow trout 24 hours after transfer to 67% seawater. This correlation, however, was over a range of 10-35 grams, and was lost when a narrower range (15-25 g) was examined. Additionally, as mentioned previously, there has long been a strong association between increased body size and improved electrolyte regulatory capacities in salmon transferred to seawater (reviewed by Wedemeyer, 1980;Folmar and Dickhoff, 1980,1981;Folmar *et al.*, 1982).

Results from the second seawater exposure experiment (72 hrs) also demonstrated a significant correlation between plasma Na^+ and plasma GH levels. However, in this experiment, the correlation was very weak and negative. The negative relationship

between the plasma GH and plasma Na^+ titers in this experiment may arise due to the return of plasma Na^+ to control or basal levels whereas the plasma GH titers continue to be elevated over the entire course of the sampling. Examining the relationship between these two parameters over the first phase of the adapation (ie., 0 to 36 hours) fails to expose any significant correlation, postive or negative. As in the first exposure, there were no correlations between body size (length or weight) and either plasma GH or Na^+ titers. Again, the narrow range of weights and lengths utilized within the experiment may preclude finding such relationships. The lack of interrelationship between body size and adaptation (ie., plasma Na^+ regulation) seems at odds with the proposed size dependency associated with smoltification (Chapter 1). McCormick and Naiman (1984b), working with brook trout, found body size was the primary factor in SW survival of these fish, but that this parameter decreased in importance in fish larger than 14 cm. The authors suggested that size in these opportunistic migrators is a primary factor but that, in anadromous *Salmo* and *Oncorhynchus* spp. (ie., those which undergo smoltification), biochemical/physiological adaptations may override size considerations. Johnston and Cheverie (1985) noted size dependency in anadromous rainbow trout also decreased in importance in fish over 11 cm. in length. Clarke and Nagahama (1977) noted that successful SW adaptation was achieved by fish which were also 1/2 the size of fish which stunted in the previous year. They proposed stunting (and, therefore, adaptation) was not a function of size, but rather of slow growth (Clarke and Nagahama, 1977)(and, thus, development)(see also Varnavskiy and Varnavskaya, 1984). As proposed in Chapter 1, differences in genetic and environmental backgrounds may override absolute size as a primary component for smoltification and, apparently, seawater adaptation.

It is very interesting that the increase in plasma GH levels was rapidly followed by decreasing plasma Na^+ titers in both experiments, ie., the delay in elevated GH levels in the second exposure was also seen in a delay of the regulation of the plasma Na^+ concentrations to basal or FW levels. This temporal relationship between plasma GH levels and plasma Na^+ regulation suggests an important role of GH in the regulatory phase of seawater adaptation, either via a direct action gill ion-pump system or indirectly, via increased gill metabolism or energetics. Folmar and Dickhoff (1980) suggest 3 possible mechanisms by which the gill Na^+,K^+-ATPase is stimulated upon entry to seawater. The first mechanism involves the existence of two functional alleles, one operating in fresh water and one in seawater. Little support for this proposed mechanism exists in the literature. Towle *et al.* (1976) was unable to detect the presence of isoenzymes from the gills of *Callinectes sapidus*, a euryhaline species of blue crab. Conte and Morita (1968) were also unable to show the appearance of isozymes in chinook salmon adapted to SW for 30 days. The second mechanism proposed by Folmar and Dickhoff postulates the existence of multiple site enzymes which, due to membrane conformations, are not active in freshwater. Most studies demonstrating increased activity of Na^+,K^+-ATPase enzymes base their results on total ouabain binding to gill homogenates. Ouabain in itself does not distinguish active versus inactive enzyme (Hughes, 1980). Additionally, the extraction or homogenation procedure involves disruption of the cells, which may also eliminate the ability to elucidate 'active' over 'inactive' (ie., exposed versus hidden) sites. Nevertheless, there have been several studies which suggest this type of model may non-anadromous euryhaline fish, which face rapid and constant changes in environmental salinity. Towle *et al.* (1977), utilizing killifish, found changes in specific activity levels of gill Na^+,K^+-ATPase within 1/2 hour of change in the external salinity. Further, they found no net increase in the ouabain binding sites

during adaptation of this euryhaline intertidal fish to 33 ppt. SW. Conversely, Hossler (1980), working with the euryhaline mullet (*Mugil capito*), reported a 6-fold decrease in ouabain binding during adaptation to freshwater.

The third hypothesis suggested by Folmar and Dickhoff proposed increased Na^+,K^+-ATPase activity is achieved via synthesis of new enzyme protein (ie., inducement of the gene, promoting synthesis of the mRNA and associated protein). Conte and Lin (1967) demonstrated *de novo* synthesis of gill epithelial protein required 4-6 days in coho. This time frame agrees temporally very well with the 3-5 day increase in gill Na^+,K^+-ATPase activity noted for coho salmon transferred to seawater (the regulatory phase)(Giles and Vanstone, 1976a;Dickhoff *et al.*, 1977;Folmar and Dickhoff, 1978,1979;Folmar *et al.*, 1980a). Conte and Morita (1968) demonstrated a 4-8 fold increase in microsomal antigenicity/units protein in 15 g chinook salmon acclimated to seawater for 30 days. This increase in antigenic response was abolished by trypsin, pointing to induction of protein. Again, the authors were unable to show the appearance of new antigens (ie., isozymes) but, rather, an increase in concentration of existing antigens over the freshwater state. Previous work by Conte (1965) had shown that X-ray irradiation of coho salmon gills destroyed the sodium regulatory capacity (no effect in freshwater sodium regulation). Further, Maetz (1969) demonstrated that treatment with actinomycin D, a transcription inhibitor, decreased Na^+ efflux in seawater-adapted fish, and Motais (1970) found actinomycin D and puromycin (an inhibitor of cellular translation) both repressed the induction of Na^+,K^+-ATPase in the gills of euryhaline eels following seawater transfer. These studies all point to genetic transcription and/or translation events inducing sodium effluxes in salmon undergoing SW adaptation, which may be reflected in the elevated levels of GH.

As yet there have been no reports of direct enhancement of gill Na^+,K^+-ATPase enzyme synthesis by GH, either *in vivo* or *in vitro*. McCormick and Bern (pers. comm.) were able to stimulate both Na^+ efflux and enzyme activity with GH in gill tissue culture, but the effects are not consistent. Boeuf *et al.* (pers. comm.) further demonstrated that 5 days of pre-treatment with GH enhanced the regulatory capacity in perfused head experiments with rainbow trout and Atlantic salmon. The development of a reliable technique for the hypophysectomy of juvenile coho salmon (Nishioka *et al.* , 1987) has allowed for some replacement-type studies. A consistent result of hypophysectomy in coho smolts is high mortality rates in full-strength seawater, due to impaired osmoregulatory functions (Bjornsson *et al.* , 1987;Nishioka *et al.* , 1987;Richman *et al.* , 1987). Bjornsson *et al.* (1987) further demonstrated that both GH and cortisol stimulated Na^+,K^+-ATPase activity in hypophysectomized salmon, whereas thyroxine treatment decreased ATPase activity. Richman *et al.* (1987) were unable to find stimulatory effects of GH treatment on gill Na^+,K^+-ATPase activity, but were able to show both increased survivability and sodium regulation in GH-treated fish.

In addition to these studies on hypophysectomized fish, earlier studies have been conducted on 'stunts', fish which demonstrate very poor survival and growth in seawater, but which do seem capable of eventually regulating electrolyte balances (Clarke and Nagahama, 1977). Fryer (1979) found GH receptors existed at the gill level in rainbow trout and chinook salmon. When these receptors were examined in coho stunts (Fryer and Bern, 1979), it was found that their ability to bind GH was impaired. Bolton *et al.* (1987b) reported that, in coho stunts held in SW for 4 months, plasma GH levels were 4-5 fold higher than in either FW or SW smolts. Also, these authors found that pituitary GH levels were significantly higher in the stunts than in the SW or FW smolts. Clarke and Nagahama (1977) also noted

that the somatotropic cells of coho stunts which had been in SW for several months, also had very high levels of GH. Additionally, they reported that these stunted fish also had a higher proportion of somatotroph cells in the PPD of the pituitary gland than the normal SW-adapted fish. These studies indicate the stunting observed in salmon transferred prematurely to seawater may be the result impaired binding of GH at the tissue levels, ie., the result of absent or inactive membrane receptors. As mentioned in Chapter 1, a similar metabolic dysfunction in humans leads to reduced growth and a condition known as Laron dwarfism. Indications of the importance of GH in seawater adaptation have also been found in other euryhaline but nonanadromous fish. Hypophysectomy of killifish, *F. kansae*, decreased the ability to secrete Na^+ in initial stages of seawater adaptation (Stanley and Fleming, 1966). Hypophysectomy also reduced gill Na^+,K^+-ATPase activity following seawater transfer of *F. heteroclitus* (Epstein *et al.*, 1967). It seems, then, that there exists ample indirect evidence for an important and complex role for somatotropin in the iono(osmo)-regulatory mechanism of seawater adaptation, and that the results of my experiments provide the first direct evidence that seawater adaptation and growth hormone are intertwined.

Although both injection groups (saline and SRIF) in the second experiment had slightly higher plasma Na^+ levels than controls at 6 hours post-exposure (with the SRIF group significantly higher than controls), it does not appear that either treatment had an overall significant effect on Na^+ fluxes in the fish. It is possible that sampling at 6 hrs post-injection may have been too late to observe any real effects of SRIF. Sheridan *et al.* (1987) noted that intraperitoneal injection of SRIF (4 and 8 ng/g) led to rapid, enzyme-mediated changes in plasma glucose and FFA levels, which were no longer apparent by 5 hours post-injection. However, the larger dose used in my

experiment was effective in lowering plasma GH levels at 6 and 12 hours post-injection, which suggests a significant amount of SRIF was present in the circulatory system at 6 hours. The results of these injections, therefore, suggest that somatostatin itself had no effect on plasma sodium fluxes early in saltwater adaptation, nor did suppression of GH release from the pituitary lead to alterations in sodium fluxes at 6 or 12 hour post-exposure. Since the data from the 24-hour experiment and the 72-hour experiment suggest GH is not involved in the early, adjustive phase of seawater adaptation, it is not suprising that suppression of GH was apparently without effect (on mortality or Na^+ regulation). It is unfortunate that there was a slight (6 hr) increase in the adjustive phase of the second experiment, else an interactive effect may have been noted as the regulatory phase began. Future experiments will hopefully provide support for this possibility. The fluctuations in plasma Na^+ seen in the FW fish may be consistent with the diel cycles seen in brook trout by McCormick and Naiman (1984a) and in killifish by Hannah and Pickford (1981). The rhythms in electrolyte balance may in turn reflect diel alterations in activity and behaviour.

The lack of any significant changes in plasma glucose during the 24-hour 'crisis' period of seawater adaptation in either the short-term or the long-term experiment would seem to indicate that this compound is not implicated during the passive 'adjustive' phase, as one might expect given the lack of increased energetic demand during this period. It is somewhat surprising that the presumed increase in plasma cortisol in this early phase of adaptation did not affect either the plasma glucose nor the tissue glucose (glycogen) compartments. In vertebrates, including fish, this hormone is considered to be both hyperglycemic (Butler *et al.*, 1969;Freeman and Idler, 1973;Chan and Woo, 1978;Suarez and Mommsen, 1987) and gluconeogenic (Fryer, 1975;Chan and Woo, 1978). While unable to

note gluconeogenesis, Freeman and Idler (1973) found that cortisol treatment of rainbow and brook trout increased the activity of hepatic glutamic-pyruvic transaminase, an enzyme involved in the energetic catabolism of amino acids. However, Abo Hegab and Hanke (1986) were also unable to demonstrate cortisol-associated hyperglycemia in 50-gram rainbow trout transferred to 30 ppt seawater, whereas when the stenohaline carp and the euryhaline tilapia were transferred to SW, elevated cortisol levels were associated with hyperglycemia. Lack of response in the second experiment may also have been due to the low liver glycogen levels in the SW group at the onset of the exposure. Nakano and Tomlinson (1967) found that rainbow trout with low initial levels of liver glycogen (≃2.5%) did not display the hyperglycemia following physical disturbance as seen in trout with higher initial levels (≃7.0%) of liver glycogen. While liver glycogen levels were not assayed in the first SW experiment, it does not seem likely that these fish also experienced such different liver carbohydrate stores at the pre-exposure period. The reason for this disparity between groups in the second experiment is unknown. It is possible, however, that the low initial plasma GH levels seen in FW control group enhanced synthesis/storage of glycogen in the liver. Smoltification is characterised by depletion of body glycogen stores (see Chapter 1) and the levels seen in the FW fish of this second experiment are very much within the normal range of values reported for fish, ie., no apparent depletion. In this regard, the subsequent (ie., post-36 hour) declines in both plasma and liver glucose levels in the SW-adapting fish would seem to be representative events. On the other hand, some of the stability in circulatory glucose titers over the initial 24-30 hrs may also be a reflection of the general 'secondary' aspect of carbohydrates in intermediary metabolism in fish. As discussed in Chapter 1, protein comprises the primary energy component in fish metabolism (Cowey and Sargent, 1977;Walton and Cowey, 1982;Weatherly and

Gill, 1987), although hatchery fish may ingest considerably higher dietary carbohydrate levels than wild fish.

In the second exposure experiment, plasma Na^+ levels in the SW uninjected fish correlated with plasma (rather than liver) glucose. This relationship may reflect the use of energy (derived by oxidation of glucose) by osmoregulatory tissues as the fish attempts to regain freshwater levels of plasma Na^+ and other electrolytes. Thus, as plasma Na^+ declines from its hypernatremic level (from 24 to 36 hours post-exposure), hypoglycemia soon follows (along with the depletion of liver glycogen stores and liver free glucose titers). Woo *et al.* (1978) also reported hypoglycemia in coho smolts adapted to SW for 6 weeks. Chinook salmon, on the other hand, which do not appear to undergo smoltification, exhibited hyperglycemia in the same study. Hogstrand and Haux (1985) reported lower whole blood glucose in 2-year-old *Salmo trutta* 24 hours after transfer to seawater, whereas whole blood lactate levels did not change. Transfer of both stenohaline carp and the euryhaline tilapia (Abo Hegab and Hanke, 1984), in addition to extended cortisol elevations, also exhibited hyperglycemia for several days. The lack of correlation between plasma glucose and Na^+ in the first experiment was probably due to the lack of any change in the plasma glucose compartment in the sampling period, again suggesting that glucose may be utilized to regulate Na^+ levels. Whether GH, alone or in combination with some other hormone(s), mobilizes carbohydrates in seawater is unknown. However, the decline of the hepatic glycogen levels during the course of adaptation suggests that glucose is being utilized at higher rates, at least during the regulatory phase. Woo *et al.* (1978) also reported that the hypoglycemia seen in coho smolts adapted to seawater for one month was not observed stunted coho smolts. It seems, therefore, that increased utilization of stored carbohydrates may be a component of SW adaptation, although the importance of this

compound remains unknown.

Although teleosts as a whole do not seem to be as dependent on carbohydrate metabolism for oxidative energy as are higher vertebrates, changes in plasma glucose could be reflective of altered utilization and/or activation of specific metabolic pathways in the tissues as well as general energetic purposes. Na^+,K^+-ATPase itself consists of two subunits, one of which is a 40,000 dalton glycoprotein (Hughes, 1980;Sargent *et al.*, 1980). The increased production of this enzyme associated with SW adaptation in salmonids would, therefore, require glucose supplies. Additionally, the chloride cells of many euryhaline fish are characterized by high levels of mucopolysaccharides. These compounds, originating in the Golgi complex, are found in much higher concentrations in SW adapted fish, predominantly associated with the greatly expanded vesiculotubular system near the apical crypts of the chloride cells (Pisam *et al.*, 1980;Pisam, 1981). Again, increased production of these compounds during SW adaptation would require increased supply of glucose. Interestingly, some studies have demonstrated positive effects of GH on mucopolysaccharide production in teleosts (Ash, 1977;Marshall, 1979).

In the 72 h experiment, plasma sodium levels in the FW fish were positively correlated to free glucose levels in the liver tissue. In all of these experiments, the level of free glucose in the liver a reflection of glycogenolysis plus gluconeogenesis. As the fish were not fed during the experiments, dietary glucose sources were nonexistent. The lack of any correlation between liver glycogen and free glucose in the freshwater fish suggests that gluconeogenesis may be more important. The strong correlation between liver glycogen and ratio (free glucose to glycogen) is, in part, due to simple mathematics (ie., liver glycogen is present in both aspects of the correlation). However, sources of free glucose in the tissue are not restricted to

glycogen as both lipids and amino acids may be directed towards gluconeogenesis. The lack of any increase in liver glycogen in the SRIF group, as opposed to the large increase seen in the saline group, was supportive of the literature which suggests somatostatin is glycogenolytic. Sheridan *et al.* (1987) demonstrated that while both somatostatin and urotensin II are glycogenolytic in coho salmon hepatic tissue *in vitro*, the former is hyperglycemic whereas U II seems to funnel glucose to fatty acid synthesis. Thus, the glucose (0.5 mg/bolus Cortlands) which apparently was incorporated into liver glycogen stores in the saline group, appears not to have been incorporated into hepatic or muscle glycogen within the somatostatin injected group. Although there was a trend towards a larger free glucose to glycogen ratio in the SRIF fish, there were no significant increases in this parameter nor in the free glucose titer of the hepatic tissue in this group. There was a slight nonsignificant increase in plasma glucose at 6 hours post-injection in the SRIF group, however this does not appear to be sufficient to account for the entire glucose injection. The exogenous carbohydrate therefore must have either been lost (eg., secreted into the urine) which seems unlikely, or converted to fats or amino acids, perhaps mediated by cortisol. Somatostatin, in addition to elevating plasma cortisol levels (Langhorne, 1986), has been shown to inhibit secretion of insulin from the β-cells of the pancreas in coho salmon, *in vivo* (Plisetskaya *et al.*, 1986). While this decrease in circulating insulin was accompanied by depletion of liver glycogen and lipids, they did not find any effect on plasma glucose titers. The decline in plasma insulin was limited to 2 hours post-injection, utilizing similar dose levels as used in my experiment. Anti-somatostatin antibody resulted in increased plasma insulin as well as increases in liver glycogen (Plisetskaya *et al.*, 1986), suggesting the somatostatin effect is a direct action on the pancreatic β-cells. McKeown *et al.* (1975) noted that injections of bovine growth

hormone led to increases in liver glycogen, again without parallel effects on plasma glucose. However, Walker and Johansen (1977) noted that hypophysectomy of the goldfish, *Carassius auratus*, led to higher liver glycogen, primarily due to decreases in glucose-6-phosphatase activity (ie., decreased glycogenolysis). Again, changes in liver glycogen levels were not reflected in the plasma glucose concentration.

Where the major role of glycolysis in hepatic tissue of fish is to provide precursors for biosynthesis of various compounds, in the muscle tissue, glycolysis is directed towards energy production. In fish, 80-90% of the total muscle mass consists of white muscle,which is primarily anaerobic, hydrolyzing pyruvate to lactate instead of directing this compound to further oxidation via Krebs cycle. While there is little evidence for gluconeogenic pathways in teleost muscle, the lactate is intracellularly recycled without having to be transported to the liver. White muscle is primarily utilized for energetically demanding 'burst' swimming in teleosts, similar to its functions throughout the vertebrates. Although capable of rapid contraction, this tissue is unable to sustain long periods of activity, due to its inefficient ATP production. It is not surprising then, that there is little glycolytic activity demonstrated by this tissue over the 72 hours of sampling, as evidenced by the stable glycogen levels. Further, many teleosts (and salmon, in particular) store large amounts of lipids in the muscle. These depots have been proposed to provide large, easily accessible energy stores for this tissue (Cowey and Sargent, 1977;Plisetskaya, 1980). Studies have shown that the adaptation of salmonids to seawater is characterized by sedentary activity, including low oxygen uptake and respiration. Flagg *et al*. (1983) suggest that some of the decreased swimming upon SW entry may be due to ionic imbalances acting to inhibit the neuromuscular junction. Further, it would be maladaptive for the fish to have

large bursts of swimming activity during these initial periods of adaptation, not only from additional metabolic energy costs, but also due to increased influxes of electrolytes across gill surfaces due to increased water flow. Fish red muscle, on the other hand, is aerobic in nature, with pyruvate being oxidized via Krebs cycle. Red muscle is the tissue used for the sustained basal swimming patterns. Unfortunately, the total mass of this tissue in most fish is quite small, being restricted to a narrow superficial band running laterally down the side of the animals. Considering the size of the fish used in my experiments, it was not possible to assay for glycogen levels in this tissue. Again, the patterns reported in the literature would suggest that there would be little change in the overall glycogen metabolism in this tissue. While muscle in fish adapting to hypersaline water has been shown to undergo dehydration, this decrease in intracellular water is usually not sufficient to significantly alter the concentration of glycogen.

It is interesting that the hematocrits in the uninjected fish in both the 24- and 72-hour experiments did not show any decreases in hematocrit over the course of the sampling, as this seems to be a common theme in seawater adaptation (Virtanen and Oikari, 1984). In steelhead trout, Houston (1964) noted a dehydration of the intracellular volume by 10%. While insufficient to cause osmotic problems within the tissues, this movement of water led to a 45% increase in the extracellular volume, providing a vital mechanism for the maintainence of circulatory volumes as well as for reduction of osmotic stresses. Both injection groups in the second experiment demonstrated a decline in hematocrit which, in the saline injected animals, was significant by 12 hours post-exposure. While it is possible that some of the hemo-dilution seen in these animals may be due to the injection volume itself (0.5 ml), Redding and Schreck (1983) demonstrated that coho stressed in FW tend to gain water and lose

electrolytes, whereas the reverse occurs in hypertonic media. Numerous studies by Schreck and his colleagues have also demonstrated elevated plasma cortisol in response to handling stress, as would be the case in both injection experiments. Thus, it seems possible that the stress of the injections, done while the fish were still in freshwater, may have had some adverse hematal effect(s).

Higgs *et al*. (1977) noted that GH treatment of coho salmon juveniles significantly increased muscle water content. Mammalian GH injected into intact rainbow trout also resulted in increased tissue water (Henderson and Chester-Jones, 1972). Conversely, Foskett *et al*. (1983) noted that hypophystectomy often leads to general cell dehydration. Clarke *et al*. (1977) suggested that water influx was not a component of the increased Na^+ regulatory capacity in GH-treated smolts, but they based their calculations on changes in total body water content, which was only on the order of 1-2%. Thus, as prolactin in freshwater may act to directly affect membrane permeability to water (Hirano and Mayer-Gastan, 1978), growth hormone may also act in some fashion to affect intracellular water volumes, perhaps through effects on intracellular concentrations of potential osmolytes (eg., amino acids). Interestingly, while LeRay *et al*. (1981) observed muscle dehydration in 100 gram trout transferred to seawater, the same was not noted in 13 gram trout (Bath and Eddy, 1979). Again, this contradictory result may derive from different degrees of smoltification/desmoltification as much as from different sizes, but it is generally considered that juvenile fish possess higher circulating levels of GH than older individuals (Grau *et al*., 1986).

Results from the first (24 hour) seawater experiment show no differences in plasma total free amino acid concentration between freshwater controls and seawater fish. The peak exhibited by both groups at 6 hours post-transfer may be indicative of either a

circadian-type rhythm or a feeding-entrained response, although there does not seem to be reports of either in fish in the literature. Conversely, the increased plasma amino acid level may be the result of sampling stress-induced cortisol release. Cortisol enhances muscle protein catabolism and subsequent release of amino acids into the circulatory system, primarily to provide fuels for gluconeogenesis in the liver. A preliminary HPLC examination of plasma amino acid profiles in the 72 h experiment (data not presented) suggested that the SW fish had higher total plasma amino acid concentrations over the entire sampling period, with glutamate, threonine, tyrosine, valine, isoleucine, and leucine generally being in greater levels in plasma of the SW fish but alanine and methionine being lower.

Several authors have proposed the utilization of free amino acids as intracellular osmolytes in muscle tissue during the adjustive phase of seawater adaptation. Lange and Fugelli (1965) found that, in the flounder (*Pleuronectes flesus*) and the stickleback (*Gasterosteus aculeatus*), intracellular tonicity in response to increased extracellular osmolality was maintained largely by organic molecules and that muscle concentrations of free ninhydrin positive substances and TMAO (trimethylamine oxide) were lower in those fish transferred to freshwater. Kaushik and Luquet (1979) found much higher total free amino acids in muscle tissue in seawater vs. freshwater trout. As well, the ratio of essential/nonessential amino acids was higher in whole blood but lower in muscle of seawater trout. Jurss *et al.* (1983) found that free amino acids in white muscle of rainbow trout contributed 25 mOsm/Kg H_2O in freshwater, 40 mOsm/Kg H_2O in 20 ppt. seawater, and 56 mOsm/Kg H_2O in starved seawater trout. In particular, the authors found the nonessential amino acids glutamate, aspartate, alanine, and serine doubled in concentration, whereas the essential amino acids were relatively unchanged. They also suggested that the further increase in

starved seawater trout (in freshwater, starvation decreases muscle amino acid levels) was due to the further osmotic load incurred by the starvation-induced reduction in gill Na^+, K^+-ATPase (Jurss *et al.*, 1983). Assem and Hanke (1983) noted that, in tilapia, taurine (the sulphonated analog of alanine) represented greater than 50% of the total free amino acid content in muscle. As well, the authors noted that the maximal dehydration of muscle tissue following seawater transfer corresponded with maximum intracellular concentration of taurine and glycine, both actively taken up from the serum. LeRay *et al.* (1981) noted that transfer of 2-yr-old rainbow trout to 32 ppt. seawater led to a rapid (within 4 hrs) increase in the muscle free amino acid pool. This increase peaked at 16 hrs. post-exposure, and had returned to freshwater levels by 31 hours post-transfer. Unlike the previous authors however, LeRay *et al.* found maximum dehydration of the muscle tissue corresponded with a peak of intracellular K^+, both peaks occurring at 31 hours post-transfer. Chloride cells of the gills demonstrate an increase in amino acid uptake from the extracellular pool in response to increased environmental osmolality (Shivakumar and Jayaraman, 1984 tilapia; Holtzman and Schreibman, 1970 platyfish). Unlike the muscle cells, uptake of amino acids by the gill chloride cells is an indicator of increased protein synthesis (Girard *et al.*, 1976). As mentioned, cortisol, in its role as a gluconeogenic hormone, increases the the rate of muscle proteolysis to provide more amino acids for the hepatic pool. It is possible that the proteolytic actions of cortisol acts to increase muscle intracellular free amino acid pools without the subsequent release of the amino acids into the circulation. Abe and Ohmama (1987) also found that starvation of 60 gram rainbow trout led to rapid large decreases in the essential amino acid *L*-histidine in white muscle, whereas starvation in addition to increased environmental salinity led to a 5-fold increase in muscle histidine content. However, the increase in this amino

acid contributed only 4% to the overall 2-fold increase in total amino acid concentration, with the bulk increase attributable to glycine (46%) and alanine (19%). Interestingly, despite these high levels of amino acids in the muscle tissue, most studies have shown decreased protein synthesis in this tissue in early seawater adaptation (Guillaume *et al.*, 1984;Nichols and Weisbart, 1985). Further, Woo *et al.* (1978) found coho salmon stunts had higher muscle protein than FW parr or SW smolts, suggesting an impaired protein metabolism may play a role in stunting. Fryer and Bern (1979) found that stunts, while possessing similar plasma GH profiles as smolts (Bern, unpub. data), had lower tissue binding capacity for growth hormone.

In summary, the literature suggests that, in the early, adjustive phase of adaptation, as the plasma osmolality increases due to the influx of electrolytes, the osmolality of the extracellular space also begins to increase as the electrolytes move into this space. In response, the intracellular space dehydrates (the water moving outwards) and the cells begin to actively uptake amino acids to act as intracellular osmolytes. As the regulatory phase of adaptation commences, the plasma electrolyte concentration drops, and the extra- and intracellular osmolalities are adjusted to their new physiological levels. This model is strikingly similar to the one proposed by Houston (1959) for the adaptation of steelhead trout to seawater. Increases in muscle water content following GH treatment were noted in sockeye (Clarke *et al.*, 1977) coho salmon (Higgs *et al.*, 1976, 1977). Perhaps GH also acts to mediate this recovery in intracellular osmolality. Uptake of amino acids, as discussed previously, is also a well-known aspect of GH actions, although I am unaware of any reports on the preferred uptake of specific amino acids as mentioned in the above reports. Unfortunately, the amount of plasma available to assay for amino acids utilizing the HPLC technique was not sufficient to provide meaningful data to

support the existence of similar movements in my studies. I hope to re-examine this aspect in future studies.

Further proof of the passive aspect of the initial, adjustive phase of seawater adaptation in salmonids was seen in the plasma FFA levels in the second exposure experiment. Whereas the FW fish begin to exhibit significant decreases in plasma total FFA levels 36 hours into the experiment (due, presumably, to food deprivation), the SW fish act to maintain plasma levels throughout the entire sampling regime, resulting in homeostatic levels of FFA during the regulatory phase. Unlike the carbohydrates, teleosts are readily able to utilize stored lipid for fuels/metabolism. Starvation in teleosts leads to rapid and preferential utilization of lipids from visceral and muscle depots in oxidative (energy-producing) pathways. Idler and Bitners (1958) noted that while migrating adult salmon tended to conserve or even increase hepatic glycogen stores, some 90% of muscle lipid and 20-30% of muscle protein was depleted in fish at the spawning grounds. Although starvation should generally lead to increases in plasma FFA levels, Farkas (1967) also noted that short-term food deprivation of the FW bream led to decreased plasma FFA levels for the first 6 days. Robinson and Mead (1973) reported that, despite significant decreases in muscle lipid content, plasma FFA levels were not elevated in starved rainbow trout, even after 5 weeks of deprivation. Responses of plasma lipid to starvation in fish are extremely variable in the literature, and are probably a reflection of a variety of diet, temperature and other conditions between the numerous studies. The change in plasma FFA in the 72 hour experiment again occurs subsequent to the rise in plasma GH. This temporal relationship points to a GH-mediated lipid mobilisation. As discussed in Chapter 1, GH acts as a lipolytic agent, increasing the release of FFA from the tissue depots to the plasma. Sheridan (1986) demonstrated that, in yearling coho, GH directly enhanced

lipolysis *in vivo* by stimulating the triacylglycerol lipase enzyme activity. As the GH-enhanced lipolysis in mammals has been shown to be a result of *de novo* synthesis of lipolytic enzymes via RNA transcription and translation (Fain, 1980), it would be interesting to examine whether the lipid mobilizing effects of GH in salmon smolts (Sheridan, 1986) can also be blocked by inhibitors of RNA and/or protein synthesis. This mode of action of GH could, in fish, display considerable temporal displacement. Further, the maintenance of plasma FFA levels in the SW group despite food deprivation does show considerable lipolytic activity. Additionally, while FFA represent a very dynamic component of lipid metabolism, the major proportion of circulating lipids is transported via lipoprotein complexes (Kayama and Iijima, 1976). Examination of this component during SW adaptation may shed further light on interaction of GH and lipid metabolism at this time.

In both classes of FFA (ie., saturated and unsaturated), there was a noticeable dichotomy in plasma profiles. In both the FW and the SW fish, those FFA of 15 carbons or less generally declined over the 72 hours. The SW fish, however, demonstrated significant elevations in plasma levels of those fatty acids of 16 carbons or more. Thus, the elevated concentrations of palmitic (C16:0), stearic (C18:0), arachidic (C20:0) and behenic (C22:0) acids were able to offset the declines in lower carbon (12-15) fatty acids. Maintenance of homeostatic levels of unsaturated FFA was achieved by increased plasma concentrations of oleic (C18:1) and linoleic (C18:2) acids. Both linoleic and linolenic (C18:3, n-3) are considered to be essential fatty acids. As the precursor for both C22:5 and C22:6, linolenic has considerably more importance for marine fish, which use these PUFAs extensively in osmoregulatory membranes. The other unsaturated FFA, which are considerably lower in concentration, were very similar in profile between both FW and SW fish over the entire sampling (although

the FW values always tended to be slightly lower than in the SW group). Palmitic acid is the major fatty acid found in fish tissues, representing up to 30% in the common pool (Plisetsakya,1980), so it is not surprising that lipolysis lead to increased levels of this compound in the plasma. Stearic acid, on the other hand, usually comprises a much smaller proportion than reported in my studies. Greene and Selivonchick (1987) state C18:0 is usually formed by *de novo* synthesis. It is an important fatty acid, as it is the precursor for C18:1(n-9) (Oleic acid) and C20:2(n-9) (eicosadienoic acid). The high levels found in my fish may reflect high dietary sources, as diet has been shown to strongly affect fatty acid profiles in both the plasma and tissues of teleosts (Cowey and Sargent, 1977; Greene and Selivonchick, 1987).

The hydrolysis and release of lipids from visceral stores, in addition to oxidative energy production and protein sparing effects, are also vital to the maintenance of the phospholipid composition of the cell membranes. If the regulatory phase of seawater adaptation is characterized by chloride cell proliferation and differentiation, then the mobilization of lipids may play important roles in all three of these aspects. First, as an energy source, lipids provide a greater efficiency (in terms of energy gain in Kcal/gm) than either proteins or carbohydrates. Weatherly and Gill (1987) suggest that oxidation of lipids (fatty acids) yields >9.0 Kcal/gm as compared to <5.0 Kcal/gm of either protein or glucose in teleosts. Thus, the large energy requirements associated with the regulatory phase of adaptation may demand utilization of these energy-rich compounds. Further, the glycerol component of lipolysis has been shown to be very important in hepatic gluconeogenesis (Plisetskaya, 1980), providing glucose for various tissues for oxidative or metabolic pathways. Maintenance of plasma FFA levels in the face of this increased metabolic activity further suggests considerable

activity of the triacylglycerol lipase enzymes. The second aspect of lipid utilization may also be vital for successful seawater adaptation. By 'sparing' amino acids from oxidative degradation, lipids redirect these building blocks for the large increase in protein synthesis associated with any hyperplasia or hypertrophy, as well as the specific increases in Na^+,K^+-ATPase enzymes throughout the various osmoregulatory tissues in the fish. Goodman and Schwartz (1974) propose this protein-sparing effect of GH (via lipid mobilization) is an important component of GH's stimulation of somatic growth. Thirdly, the released fatty acids from the lipid stores can be utilized in the specific production of phospholipids for cell membranes. Not only in this cell component going to increase as hyperplasia proceeds, but the maturation or differentiation of the gill chloride cells is characterised by an enormous proliferation of the vesiculotubular membrane system, acting to substantially increase the paracellular surface area over which Na^+ and K^+ exchanges take place (Girard *et al.*, 1976; Pisam, 1980;Foskett *et al.*, 1983). This large increase in membrane area would neccessitate increased supply of lipids for structural purposes.

In addition to changes in overall plasma FFA profiles, a number of studies have demonstrated that the activity of many membrane enzymes, including Na^+,K^+-ATPase and carbonic anhydrase, are dependent upon the composition (particularly the degree of unsaturation) of the phospholipid component of the associated membrane. Further, much of the enhanced activity of these enzymes appears to be particularly linked to increases in specific fatty acids, ie., C20:5 and C22:6. Thus, Poon *et al.* (1981) reported that plasma membrane Na^+,K^+-ATPase enzyme activity of murine T lymphocytes was inhibited by saturated fatty acids while enhanced by unsaturated fatty acids, primarily by increasing levels of docosahexaenoic acid. The authors further state that that this enhanced ouabain binding results directly from the degree of

unsaturation, rather than from the increased fluidity of the membrane. Bell *et al*. (1986) further state that, in addition to transport processes, the membrane fatty acid composition also effects such interactions as cell proliferation and fusion as well as receptor functions. They also report that, as an essential fatty acid, dietary deficiencies of C22:6 in fish lead to gross disturbances in the gill structure, including degeneration of the chloride cells. In addition to these studies, several reports note that changes in the external salinity have been demonstrated to have effects upon the composition of membranes, especially upon the osmoregulatory membranes. Thus, LeRay *et al*. (1981) reported that the transfer of rainbow trout to SW led to a marked increase in the fluidity and permeability of gill membranes. This increase was due to the very large increase in polyunsaturated fatty acid composition of the membrane phospholipids, in particular the phosphatidylcholine component. This phospholipid comprises a large proportion of gill membranes in teleosts. The increase in PUFA content in these fish was primarily due to increased C20:5 and C22:6 levels, in exchange for C18:0. DiConstanzo *et al*. (1983) reported that the transport of Na^+ and Cl^- across the intestinal membrane of SW-adapting trout was directly related the the polyunsaturated fatty acid (ie, C20:5 and C22:6) content. Zwinglestein *et al*. (1979-80) also noticed highly increased ^{35}S incorporation (ie., increased sulphatide synthesis) in seawater-adapting eels, but found no correlation between sulphatide content and Na^+,K^+-ATPase. Further, Hansen (1987) noted that increased external salinity increased polyunsaturated fatty acid content of membranes of gill, intestine, and esophagous, but had no effect on the fatty acid composition of the liver in eels. Conversely, Morris *et al*. (1987) demonstrated that transfer and adaptation of the marine amphipod *Gammarus duebeni* to dilute media led to an increase in the proportion of saturated fatty acids in the gill lipids. This change in composition was not observed in any of the

other body lipids. This selective action of external salinity on osmoregulatory membrane composition suggests that this process is not a general response to changes in environmental salinity, but is probably mediated by hormonal and neural actions. Greene and Selivonchick (1987) state that phosphatidylcholines are preferentially synthesized from pentadecanoic and hexadecanoic diacylglycerides in teleosts. They further report that the increase incorporation of C22:6 in the gills of SW-adapting eels replaces C20:4 (n-6), which is the precursor for PGE_2, a prostaglandin which inhibits salt secretion in the gills. Mobilisation of lipids from various depots within the body would provide some the polyunsaturated fatty acids or their precursors to the osmoregulatory tissues during seawater adaptation. If, as Jezierska *et al*. (1982) suggest, there is a difference in the degree of unsaturation of triglycerides in various depots, and that, under different conditions (or duration of conditions), different depots are preferentially mobilised, then the temporal separation of hormone release during SW adaptation (eg, cortisol vs. GH) may have very important effects not only on the metabolism of various target tissues, but also upon the nature of the metabolites released into the circulation for these target tissues to utilize. In addition to effects on plasma membranes composition, GH may also affect composition and, thus, function cellular organelles. In mammalian studies, GH has been shown not only to induce cytoplasmic and mitochondrial protein synthesis in hepatic tissue, but also to increase the activity of respiratory exzymes in the hepatic mitochondria (Maddaiah and Clejan, 1986). Clejan and Schulz (1986), also working with hypoxed rats, demonstrated increased incorporation of polyunsaturated fatty acids (e.g.,C22:6) into the phospholipids of hepatic mitochondria subsequent to GH treatment. The resulting altered fluidity of the mitochondrial membrane may be reponsible for the increased activity of the respiratory enzymes. These authors also demonstrated regulatory effects of GH on enzymes involved in the

formation (oxidation) of polyunsaturated fatty acids in the mitochondria (Clejan and Schulz, 1986). If similar effects occur in teleosts, then GH may play an important role in increasing the metabolic activity rates of various organs involved in the regulatory phase, such as the liver and the gills. Respiration rates in the gills of SW-adapting fish have been shown to be inhibited over the initial phase of adaptation, but to be greatly enhanced as regulation begins.

Being a pancreatic hormone in addition to its role as a GH-release inhibiting factor, SRIF is also involved in aspects of intermediary metabolism (Plisetskaya, 1986). Minick and Chavin (1970) reported serum hypolipidemia 6-9 hours post-injection of SRIF in goldfish. In contrast, Sheridan and Bern (1986) reported a lipolytic action of SRIF in coho salmon hepatic tissue, *in vitro*, further supported by hyperlipidemia and enhanced lipolytic enzyme activity in *in vivo* studies. The decreasing plasma lipid levels seen in my SRIF-injected fish disagree with the results of Sheridans' work. However, it is possible that my results represent a hypercompensation. Sheridans' results were observed at 3 hours post-injection, whereas no such results were observed by 5 hours (Sheridan *et al.*, 1987). Further, Plisetskaya *et al.* (1986) reported an anti-insulin effect of SRIF injection, followed by a compensatory hyperglycemia and hyperlipidemia. Whether these various effects and interactions have important results in the present study is unknown. Nor have there been any reports of changes in SRIF metabolism in SW-adapting fish. The unsaturated and saturated FFAs in my injected fish declined at much different rates depending upon saline alone or saline+SRIF, with the SRIF injected fish having much higher S/U ratios at 6 hours. Further experiments utilizing a more specific GH-release inhibitor may shed some light in some of these results.

In addition to GH and cortisol, thyroid hormones may also be involved in seawater adaptation. While several authors have shown

hypoactivity of the thyroid gland in seawater adapted fish, Redding *et al*. (1984b) noted an initial surge in plasma thyroid hormone levels early in adaptation of juvenile coho to seawater, with plasma thyroxine peaking at 12 and 72 hours post-exposure and plasma T_3 titers showing peaks at 12 hours and returning to control levels by 24 hours after transfer. These different increases in plasma T_3 and T_4 levels may have both direct and indirect effects on seawater acclimation. While both thyroxine and triiodothyronine have been shown to increase amino acid uptake and protein synthesis in tissues, Nayaransingh and Eales (1975) further noted that T_3 had a greater effect than T_4 on gill tissue whereas the reverse was true for hepatic tissue. Additionally, Omeljaniuk and Eales (1986) reported that, in freshwater coho, increased plasma T_3 led to depression of gill Na^+,K^+-ATPase activity while elevating hepatic ATPase levels. Thus, the plasma ratio of T_4/T_3, as determined by hepatic and peripheral monodeiodination and/or plasma protein binding (Eales, 1987), may also be an important factor in enhancing organ activity. The peripheral monodeiodination of T_4 to T_3 has been shown to be reduced by hypophysectomy and starvation in rainbow trout (Levin and Eales, 1982;Komourdjian and Idler, 1978) and by increased cortisol levels in juvenile coho salmon (Redding *et al*, 1984b). DeLuze and LeLoup (1984) and Miwa and Inui (1985) have suggested that growth hormone may act to increase peripheral conversion of T_4 to T_3 in eels and amago salmon, *Oncorhynchus rhodurus*, respectively. Conversely, injection thiourea, a potent inhibitor of thyroid hormone secretion, into *Fundulus heteroclitus* was lethal only upon transfer of the fish to seawater (McNabb, in Shivakumar and Jayaraman, 1984). These authors found similar results with *Sarotherodon mossambicus*. They also noted that simultaneous injections of thiourea and T_4 resulted in 100% survival when the fish were transferred to seawater. Thyroxine was also shown to bind directly to gill mitochondrial membranes and to enhance <u>total</u> enzyme activity in

the gills via mitochondrogenesis (Shivakumar and Jayaraman, 1984). In addition to these direct actions, thyroid hormones may be important mediators of GH effects. As discussed in Chapter 1, thyroxine increases somatotroph activity in a number of fish. Thiourea, on the other hand, decreased somatotroph activity in *Tilapia zillii* (Leatherland and Hyder, 1975).

It is apparent from my data that there is a large and persistent response of GH to increases in external salinity. The magnitude, timing, and duration of this hyper-secretion suggests that GH may be a vital aspect of the regulatory phase of adaptation as opposed to the initial adjustive phase, which, from the literature, may be the domain of cortisol. The subsequent increased mobilisation of both carbohydrates and lipids are consistent with known effects of GH on these compounds. Without kinetics or studies of metabolism at the tissue levels, however, it is difficult to ascertain whether GH acts to indirectly drive the regulatory phase via the provision of potential precursors or energetic fuels, or whether there is some direct action of GH on osmoregulatory function, such as the increased synthesis of Na^+,K^+-ATPase or increased production of the chloride cells themselves. The action of GH on the class of compounds known as proteins was not evident in either of the experiments discussed in this chapter, although the numerous reports in the literature of the possible involvement of this parameter in both direct and indirect fashion strongly suggests that the interaction between GH and protein metabolism is also a vital component of SW adaptation. The interaction of hormones, as proposed in Chapter 1 for smoltification, is probably a very important feature of successful adaptation to the marine environment. In addition to cortisol (acting in the initial phases) and GH (acting in the regulatory phase), such hormones as thyroxine, insulin, SRIF, and urotensin, may all be acting at various post-exposure times to ensure survival and growth.

GENERAL CONCLUSIONS

The large increases in plasma GH levels seen as smoltification proceeds confirm that increased secretion of this hormone plays an important role in the successful transition to a seawater fish. The increase in plasma GH was most notable as smoltification neared completion, a result very similar to plasma profiles seen for two other hormones which have long been associated with successful smoltification, ie., cortisol and thyroid hormone. Equally as important, however, was the dramatic increase in plasma GH levels seen following actual seawater entry of the smolts. These results confirm that GH not only is important in the preparatory or pre-adaptive phase but also during the actual adaptation phase. The data also indicates that one must view smoltification and seawater adaptation as separate, yet obviously linked, events in the life cycle of the salmon.

In both the chronic long-term increase seen during smoltification, as well as the short, acute response observed following seawater entry, an important function of the elevated GH levels appears to be the enhanced catabolism or mobilisation of both carbohydrate and lipid stores. This action may act to increase the circulatory fuels and to furnish energy for the increased metabolic rates characteristic of both of these phases. Also, high metabolic rates require high levels of energy-rich compounds, which, in fish, are often protein in nature. The increased availability of glucose and free fatty acids may act to spare amino acids for protein synthesis and cell growth, also associated with both events. Additionally, specific amino acids may be diverted and utilized in non-anabolic fashions, eg., glycine as a precursor for purine deposition in scales, taurine and glycine as intracellular osmolytes. On the other hand, the mobilisation of these compounds may also reflect an increased anabolic demand for either glucose or fatty acids, such as

increased production of the glycoproteinaceous Na^+,K^+-ATPase enzyme. The same may be said of inidividual free fatty acids, which, instead of undergoing mitochondrial oxidation, may be utilized as membrane phospholipids (especially C22:6) or as lipid components of newly synthesized proteins. The change of lipid composition from a freshwater type to a typically marine type during smoltification suggests that there is some differential utilization of free fatty acids, which may not necessarily be visible in plasma profiles. Growth hormone may also play some role in shifting tissue lipid composition by specifically enhancing mobilisaton of saturated fatty acids during smoltification. In either case, the results agree with known actions of growth hormone: increased rates of lipolysis, increases in glucose availability, increases in the transport and uptake of amino acids.

In both of the seawater adaptation experiments, it appeared that GH may also be acting in a direct fashion to enhance the successful transition into the marine environment. The lack of an increase in the first 12-18 hours of seawater exposure indicates that GH is not important during this initial passive phase. However, the large increase in plasma GH seen just prior to the onset of the regulatory phase, ie, just prior to the return Na levels to homeostatic values, intimates that GH may indeed be acting directly upon the gill chloride cells to initiate osmo(iono)regulation. The subsequent increases in carbohydrate and lipid mobilisation would then be acting in an indirect fashion.

Growth hormone also appears to be an important regulatory factor in desmoltification, as evidenced by the extremely high plasma GH levels, as well as the coincident perturbations in a number of plasma and tissue metabolite concentrations. These animals represent an interesting and under-utilized source of information regarding the parr-smolt transformation. In future

studies, it will become increasingly important to study these fish as well as the pre-smolts.

The lack of consistent patterns in the other plasma parameters during both the smoltification and the seawater studies point to the difficulty of following these events over different years. Variations in nutrition, endocrinological history, environmental factors, etc. will create alterations in the individual metabolic responses. Whether these variations will affect successful transition or seawater must await future more comprehensive studies.

In summary, my research has provided the first direct proof that growth hormone is involved in the parr-smolt transformation as well as in actual seawater adaptation. While I was unable to assign specific role(s) to GH in these two complex events, I feel that my results show that GH must be considered, along with cortisol and thyroid hormone, as an important regulatory factor in the successful transformation of juvenile salmon from a freshwater fish into one capable of survival and growth in the marine environment. empty -print empty -punch run *textform sprint=-print spunch=-punch

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